# Stemphylium vesicarium on Garlic and other Allium spp. in Korea

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## 마늘 및 기타 Allium속 식물에 발생하는 Stemphylium vesicarium

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ABSTRACT: More than 100 isolates of *Stemphylium* obtained from leaf blight lesions of garlic (*Allium sativum*), onion (*A. cepa*) and leek (*A. fistulosum*) were identified as *Stemphylium vesicarium* (Wallr.) Simm. based on various morphological characteristics rather than *S. botryosum* Wallr. previously reported on those plants in Korea. In controlled inoculations, the pathogen induced the disease symptoms on leaves of garlic 7 days after inoculation. Onion and leek were also artificially infected by the fungus. This is the first report of *S. vesicarium* causing a leaf blight of garlic, onion and leek in Korea.

Key words: garlic, leaf blight, leek, onion, Stemphylium vesicarium.

Garlic (Allium sativum) is one of the most important vegetable crops in Korea. Cultivation of garlic occupies over 39,600 ha with a total production of 462,000 ton per year (1). Fungal diseases such as leaf blight, rust and white rot cause severe damage to garlic when weather conditions favor the disease development (7).

During past two years, particularly in 1998, severe outbreak of a leaf blight occurred in commercial fields of garlic throughout the country. In some fields foliage losses were as high as 90%. Disease symptoms consisted of ellipsoidal to oval and brown to dark brown zonate leaf lesions. The lesions sometimes showed a purplish colour resembling to those of the purple blotch disease caused by *Alternaria porri*. The lesions turned light brown to tan and eventually killed older leaves (Fig. 1-A, B). Some of the lesions became black when spores were produced. Infection often started at the leaf tips, which withered prematurely, resulting in yield reduction. The disease was also prevalent on onion (*Allium cepa*) (Fig. 1-C) and leek (*Allium fistulosum*).

A species of *Stemphylium* was consistantly isolated from the diseased leaves of garlic, onion and leek. *Stemphylium botryosum* Wallr. is reported as the cause of leaf blight of garlic, onion and leek (2, 7). However, conidia of the fungus isolated in this study were mor-

phologically distinct from those of *S. botryosum*. This paper reports on observations of its morphology and the disease it causes.

#### MATERIALS AND METHODS

**Isolation.** Pieces of tissue from the margins of the lesions on garlic, onion and leek leaves were immersed in 1% NaOCl solution for 1 min, rinsed in sterile water and incubated on potato dextrose agar (PDA) at  $25\pm1^\circ$  C. Spores were also directly transferred from lesions on leaves to PDA and incubated under the same conditions. After 7 days of incubation, the *Stemphylium* sp. colonies were transferred to PDA slants and stored at 7 °C.

Identification. Attempts were made to identify the Stemphylium sp. by culturing on PDA and V-8 juice agar at 25°C under near ultraviolet lamps with a 12 h day-12 h night photoperiod. Microscope slides of conidia and conidiophores of the isolates were prepared from 7-day-old culture on V-8 juice agar by mounting thin slices of the sporulating culture in distilled water or lactophenol. Slides were also prepared from lesions on garlic, onion and leek leaves after incubation in a damp chamber for 24 h. The size, shape, number of transverse and longitudinal septa, lateral constrictions, surface ornamentation of conidia were recorded, and

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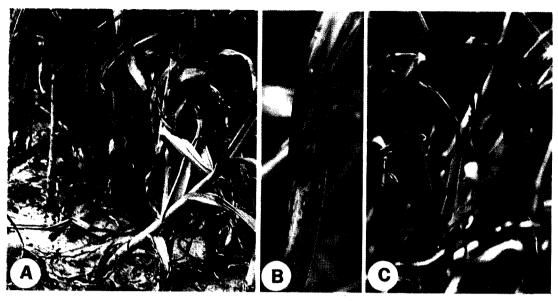


Fig. 1. Leaf blight symptoms on naturally infected garlic and onion, (A) Severely infected garlic plants in a commercial field; (B) A typical lesion on a garlic leaf; (C) Severely infected onion plants in a field.

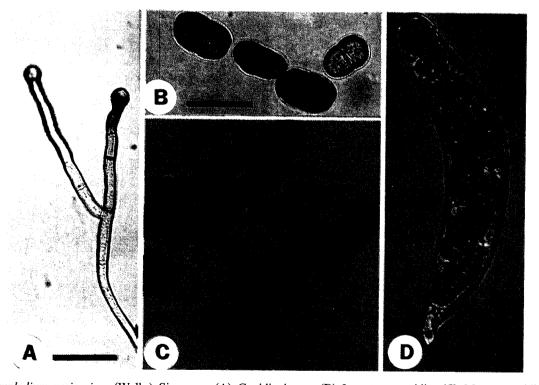


Fig. 2. Stemphylium vesicarium (Wallr.) Simmons, (A) Conidiophores; (B) Immature conidia; (C) Mature conidia; (D) Ascus containing eight ascospores. Each bar indicates  $35~\mu m$ .

the length-to-width (1:w) ratio was calculated. The isolates were also compared with the isolate of *Stemphylium vesicarium* (IMI 135457) obtained from the CABI Bioscience, England. The cultures were also grown under the above conditions until pseudothecia developed, usually after 2~4 weeks. The plates were then transferred to a refrigerator at 7°C until fully mature ascospores developed and the size of asci and ascospores of the perfect state (*Pleospora*) was measured.

Pathogenicity. For pathogenicity tests, 3-week-old

plants of garlic, onion and leek were sprayed using a hand-sprayer with 10 ml of a conidial suspension adjusted to  $1 \times 10^5$ /ml. Control plants were sprayed with sterilized water. The sprayed plants were placed in a dew chamber at 25°C for 2 days to ensure favorable conditions for infection. The plants were then transferred to a green house at 25~27°C. Disease was assessed up to 20 days after inoculation.

#### RESULTS AND DISCUSSION

Cultures of the fungus from infected leaves on PDA produced grey to greyish brown, hairy or velvety mycelia when incubated in the dark. The colonies reached 63 to 70 mm in diameter after 7 days incubation at 25 °C. The conidiophores of our isolates were erect, brown with vesicular swellings in the apices of the conidiogenous cells, 3 to 8 septa, measuring 22~170×3~10 um (Fig. 2-A), and bearing a single conidium at the apex. Conidia were oblong or broadly ellipsoidal, olivaceous brown to mild black, rounded at the apex with no pointed ends, and with the cell wall minutely verrucose. The number of transverse septa ranged from 1 to 6 with several longitudinal irregular septa. Juvenile conidia were constricted at one median transverse septum (Fig. 2-B), but oblong mature conidia commonly constricted at the two to three major transverse septa (Fig. 2-C). Average dimensions of 100 conidia obtained directly from the host were 25~53×15~30  $\mu m$  with a 1: w ratio of 1.9~2.1:1, and 25~50×12~32 μm with a 1:w ratio of 2.0~2.3:1 in V-8 juice agar

culture (Table 1). Dimensions of the isolate of *S. vesicarium* obtained from the CABI Bioscience (IMI 135457) are also presented in Table 1. The perfect state (*Pleospora* sp.) of the isolates was developed on V-8 juice agar in  $2\sim4$  months. The ascospores were brown,  $30\sim50\times12\sim23~\mu m$  (Av.  $38.5\times16.8~\mu m$ ) and often pointed at the tip (Fig. 2-D).

A comparison of the measurements of conidia and conidiophores from leaf lesions on *Allium* spp. and from cultures grown on V-8 juice agar agree closely with those published for *S. vesicarium* (Wallr.) Simmons (Table 1) (5, 9, 16, 18). Ascospores of the isolates were also similar to those of the *Pleospora* teleomorph of *S. vesicarium* described by Simmons (18).

S. vesicarium is distinguished from S. botryosum by the conidial morphology and the number of septum in mature spores (Table 1) (9,17), although both have muriform olivaceous brown conidia rounded at the apex, with no pointed ends (15, 17). Conidia of S. botryosum usually have 3 transverse and 1~3 longitudinal septa, constricted at one medium transverse septum, and with a 1:w ratio of  $1.0\sim1.5:1$  (17). In S. vesicarium the conidia have up to 6~7 transverse and several longitudinal septa, often constricted at two to three major transverse septa, and with a 1:w ratio of appoximately 2.0~3.0:1 (9, 14~16, 18). Conidia of S. botryosum are proportionally shorter than those of S. vesicarium (9, 18). Studies on more than 100 cultures isolated from lesions on garlic, onion and leek from a large number of locations in Korea indicated that in all cases the pathogen fitted to the description of S. vesicarium rather

**Table 1.** Conidial dimensions and length-to-width ratios (1:w) of *Stemphylium* isolates in comparison with those of published descriptions of *S. vesicarium* and *S. botryosum* 

Isolate	Conidial morphology			
	In culture		On host	
	Length × Width (μm)	1:w	Length×Width (μm)	1: w
Garlic isolate MSV-01	27~45×12~20(Av. 37.4×16.3)	2.3:1	25~53×15~25(Av. 40.9×22.7)	1.9:1
Onion isolate OSV-05	$27 \sim 50 \times 15 \sim 32$ (Av. $37 \times 22.6$ )	2.0:1	$30 \sim 52 \times 15 \sim 30$ (Av. $39.9 \times 19.7$ )	2.0:1
Leek isolate PSV-01	$25\sim43\times15\sim25(\text{Åv. }35.0\times19.7)$	2.2:1	$27 \sim 45 \times 15 \sim 25 (Av. 35.9 \times 20.3)$	2.1:1
S. vesicarium	•			
IMI 135457	$30\sim52\times17\sim25(Av.\ 42.3\times20)$	2.2:1	_	_
Simmons (18) <sup>a</sup>	$-\left(\mathrm{Av.}\right. < 45 \times 18\right)$	2.5~3.0:1	25~42×12~22(Av. 33.4×17.7)	1.5~2.7:1
				(Av. 1.9:1)
Irwin et al. (9)	$26 \sim 43 \times 11 \sim 19$ (Av. $35.5 \times 14.5$ )	2.3~2.7:1	_	_
S. botryosum				
Simmons (18)	$-(Av. 33 \times 23)$	1.5:1	$33 \sim 35 \times 24 \sim 26 (Av. 34 \times 25)$	1.0~1.5:1
Irwin et al. (9)	$23 \sim 33 \times 15 \sim 23$ (Av. $28 \times 18$ )	1.5:1		_

<sup>&</sup>lt;sup>a</sup>Refer to reference number.

than S. botryosum previously reported on garlic in Korea. S. botryosum was not found in the present study. The consistent occurrence of S. vesicarium on both garlic and other Allium spp. suggest that previous reports of S. botryosum in Korea were probably misidentification of S. vesicarium.

Disease symptoms on garlic, onion and leek appeared 7 days after inoculation. Symptoms on onion and leek leaves were similar to those on garlic. Control plants had no lesions. Lesions from the plants inoculated with the isolates were placed in moist chamber yielded conidia identical to the isolated *Stemphylium vesicarium*. S. vesicarium has been reported on garlic in South Africa (3), Spain (4) and Brazil (5), onion in USA (14, 16), and India (15), alfalfa in USA (6) and South Africa (12), and asparagus in USA (8, 10, 11) and New Zealand (13). This is the first report of S. vesicarium causing a leaf blight of garlic, onion and leek in Korea.

#### 요 약

마늘, 양파 및 파의 잎마름병 병반에서 분리한 100개이상의 Stemphylium 균주들의 여러 가지 형태적 특징을 조사하였던 바 이들 Allium속 식물의 잎마름병균은 그동안 국내에서 보고되었던 Stemphylium botryosum Wallr.과 구별되는 S. vesicarium(Wallr.) Simm.으로 동정되었다. 접종실험에서 이 균은 접종 7일 후에 마늘잎에 병징을 일으켰으며 양파와 파에도 병원성이 있었다. 이것은한국에서 마늘, 양파 및 파의 잎마름병을 일으키는 S. vesicarium에 관한 최초의 보고이다.

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