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Occurrence of Target Leaf Spot of Red and White Clovers Caused by *Stemphylium sarciniforme* in Korea

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A target leaf spot of red and white clovers was observed during 1998-2000 growing seasons in several fields of Chungnam and Chunbuk provinces in Korea. Lesions were circular to oval in outline, brown to dark brown, ranging from a pinpoint to 3-4 mm in diameter, often concentrically zonate and presenting a target effect. A fungus associated with the disease was identified as *Stemphylium sarciniforme* based on the morphological characteristics of the conidiophores and conidia. The fungus was pathogenic on red and white clovers in the inoculation test. This is the first record of a target leaf spot of red and white clovers in Korea.

Keywords **:** *Stemphylium sarciniforme, Trifolium pratense, T. repens.*

Stemphylium species cause a number of diseases of plants such as leaf spot of tomato (*Lycopersicon esculentum* Mill.), caused by *S. lycopersici* (Enjoji) Yamamoto (Min et al., 1995) and *S. solani* Weber (Kim et al., 1999); leaf spot of pepper (*Capsicum annuum* L.), caused by *S. lycopersici* and *S. solani* (Kim et al., 1996); and leaf blight of garlic (*Allium sativum* L.), onion (*A. cepa* L.) and Welsch onion (*A. fistulosum* L.), caused by *S. vesicarium* (Wallr.) Simmons (Cho and Yu, 1998). These diseases are serious in wet weather and under moist conditions.

During a survey of *Stemphylium* diseases of plants in Korea, a leaf spot disease of red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.) was observed during September-October 1998 in a field in Seonghwan, Chungnam province, Korea. Further survey of the red and white clovers during the 1999 and 2000 seasons in different fields of Chungnam and Chunbuk provinces revealed that the disease was widespread and was responsible for considerable damage under the moist conditions. A species of *Stemphylium* was repeatedly isolated from lesions of the plants. It fitted the description of *Stemphylium sarciniforme* (Cav.) Wiltshire (Wiltshire, 1938; Neergaard, 1945; Simmons, 1969; Booth, 1980). The objectives of this study were to record the disease, and to describe the disease symptoms on red and white clovers and the characteristics of the pathogen.

Symptoms of the disease on the leaves of red and white clovers were circular to oval in outline, ranging from a pinpoint to 3-4 mm in diameter (Fig. 1-A, B). During early stages of development, the leaf spots were small but conspicuous dark brown. As the disease progresses, the central portion of the leaf spot bleached to pale brown, leaving a well defined dark brown margin. On the older lesions, the spots often became concentrically zonate and presenting a target effect. Under severe disease conditions, the spots coalesced and produced a leaf blight.

Conidia of a species of *Stemphylium* were produced abundantly on leaf lesions when incubated in a moist chamber for 24 h. The fungus was characterized as follows: Conidiophores arising singly or sometimes in groups of 2-3, simple or branched, straight to mildly flexuous, cylindrical but enlarged slightly at 1-3 apical percurrent proliferations, pale to golden brown, septate, up to 60 × 5-10 μm with vesicular swellings of 10-14 μm diameter (Fig. 1-C). Conidia solitary, subspherical or broadly ellipsoid, rounded at both the base and apex, golden brown, smooth-walled, 22-34 (-40) × 18-27 μm with usually 3 transverse and several longitudinal septa, distinctly constricted at the median transverse septum and with a l:w ratio of 1.5:1 (Fig. 1-D). The above characteristics enabled the fungus to be identified as *Stemphylium sarciniforme* (Cav.) Wiltshire (Booth, 1980; Neergaard, 1945; Wiltshire, 1938) (Table 1). The fungus has been recorded to cause a target leaf spot on *Trifolium* spp. from USA (Anonymous, 1960; Kilpatrick, 1959; Renfro, 1960), Europe (Booth, 1980; Neergaard, 1945) and Japan (Yamamoto, 1960), but not from Korea (Anonymous, 1998).

Isolations of the *Stemphylium* were made directly from conidia developed on leaf lesions with a finely drawn glass needle under a dissecting microscope to potato dextrose agar (PDA) plates. A stock culture was prepared from the colony by transferring a mycelial plug to a PDA slant for further study.

For determining the pathogenicity of the fungus, inoculum was produced by transferring mycelial plugs from a

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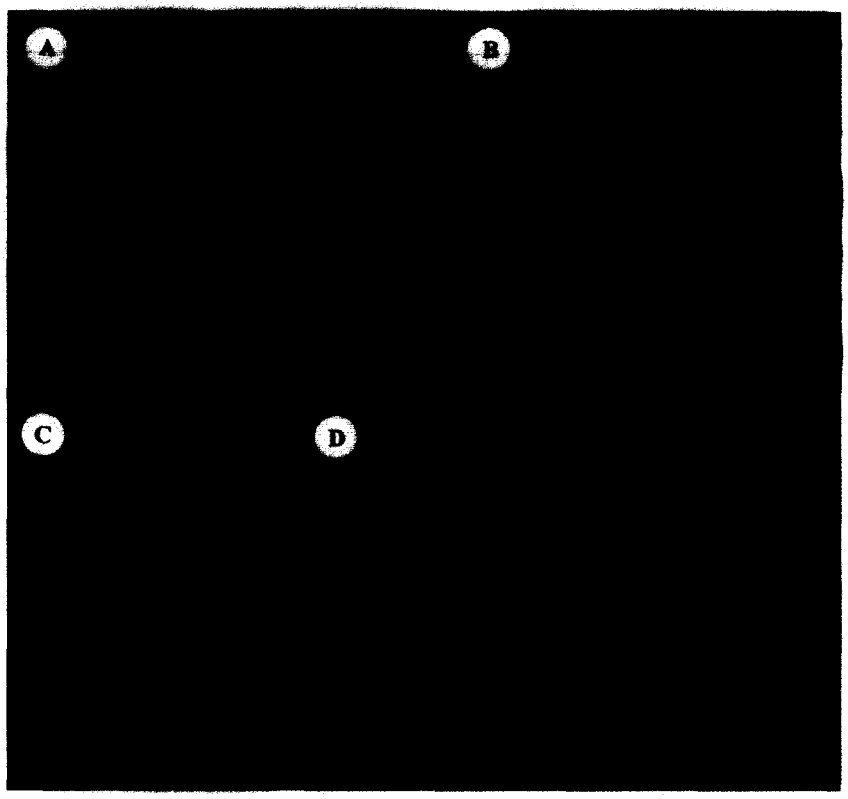

Fig. 1. Symptoms of target leaf spot on *Trifolium pratense* (A) and *T. repens* (B) caused by *Stemphylium sarciniforme*. Conidiophores (C) and conidia (D) of *S. sarciniforme* isolated from a lesion of *T. pratense*. Bar represents 50 μm.

Table 1. Comparison of morphological characteristics of conidiophores and conidia of the present isolate and *Stemphylium sarciniforme* reported by previous workers

Morphological feature	Present isolate	*S. sarciniforme*	
		Wiltshire[a]	Booth[b]
Conidiophores			
Color	Pale to golden brown		Pale to mid golden brown
Length	Up to 60 μm	16-50 μm	Up to 50 μm
Width	5-10 μm	6-8 μm	6-10 μm
Conidia			
Color	Golden brown		Golden brown
Shape	Subspherical or broadly ellipsoid	Ovate or rather elongated	Subspherical or broadly ellipsoid
Surface	Smooth	Smooth	Smooth
Septation	Usually 3 transverse and several longitudinal septa, median constriction	Median constriction	Ususally 3 transverse and several longitudinal septa, median constriction
Dimension	22-34(-40) × 18-27 μm	28-38 × 18-29 μm	30-50 × 22-33 μm

[a] Described by Wiltshire (1938)
[b] Described by Booth (1980)

stock culture to fresh V-8 juice agar. The pure cultures were incubated at 22°C in a 12 h dark/ 12 h light regime for 10 days. Conidia were obtained by flooding the cultures with 10 ml sterile water and rubbing the fungal colony with a sterile brush to dislodge the conidia.

Inoculation experiments were carried out on the detached leaves of red and white clovers. The detached leaves were inoculated by placing on each leaflet 5-10 μl drops of conidial suspension, each containing 30-50 conidia. Control leaves were inoculated with sterilized water. The detached leaves were placed on moistened spongy layer in plastic boxes, incubated at 22°C in a growth chamber. The plastic boxes were kept in the dark for 48 h and then given a 12 h photoperiod of cool-white fluorescent lighting. Typical small brown spots were observed 3 days after inoculation on the leaves. Symptom on white clover leaves was similar

to that on red clover. No visible symptom was observed on the leaves treated with sterilized water. *S. sarciniforme* was consistently re-isolated from the lesions induced by artificial inoculation.

This is the first report of *S. sarciniforme* on red and white clovers in Korea. We propose the name of the disease as 'gyeopmunibyeong' in Korean since it is shown as a concentrically zonate leaf spot. A total of seven specimens were collected, and preserved in the mycological herbarium (CMH) of Chungnam National University as follows: CMH-98022 (17 X 1998, Seonghwan), 98024 (17 X 1998, Seonghwan), 99240 (20 VI 1999, Taejon), 00106 (28 VI 2000, Seonghwan), 00107 (28 VI 2000, Seonghwan), 00108 (29 VI 2000, Namwon), 00109 (1 VII 2000, Kongju).

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