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#### RESEARCH NOTE

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## First Report of Septoria centellae Associated with Leaf Spot of Centella asiatica in Korea

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Septoria centellae associated with leaf spot of Centella asiatica is reported for the first time in Korea. The fungus is described and illustrated in detail. It is also compared with related species of Septoria present on Centella and Hydrocotyle spp. with taxonomic comments. Two monoconidial isolates from Jeju and Wando were successfully cultured and have been deposited in the Korean Agricultural Culture Collection.

KEYWORDS: Centella asiatica, Hydrocotyle asiatica, Septoria centellae, Septoria hydrocotylicola

The anamorphic fungal genus *Septoria* is one of the largest genera of plant pathogens, causing a range of disease symptoms including leaf and fruit spots in agricultural crops, as well as horticultural and native plants. It comprises about 1,000 [1] to over 2,000 species [2, 3]. As of August, 2011, the database of the Index Fungorum (http://www.indexfungorum.org) contained 3,123 *Septoria* entries including synonyms, varieties, and superfluous names.

Some monographs for certain countries have considerably contributed to the knowledge of species diversity, taxonomy, ecology, and distribution of Septoria fungi [4-6]. In Korea, Shin and Sameva [7] monographed 72 species of Septoria found on 112 species of host plants. They listed an additional 27 species of Septoria previously recorded in Korea but not treated in their monograph due to lack of herbarium materials. Nevertheless, the real mycobiota of Septoria in Korea has been insufficiently studied. Mycological studies for the Korean Septoria spp. are needed for diagnosis of the diseases caused and precise identification of the fungus. During an extensive collection of phytopathogenic fungi in Korea by Shin and colleagues, we found a Septoria on Centella asiatica (L.) Urban (= Hydrocotyle asiatica L.) for the first time in Korea. In this report, we describe and illustrate this fungus with taxonomic comments.

Field observations including symptoms on host plants were described for taxonomic reference according to previous studies [5, 7]. Morphological characteristics of the fungus were ascertained by light microscopic examination using a model BX51 microscope (Olympus, Tokyo, Japan), based on a representative sample (KUS-F25398). Images were recorded using a Zeiss Axio imager microscope (Carl Zeiss, Göttingen, Germany). Examination of fungal structures was based on fresh materials. In each case, 30 measurements of structures of taxonomical value were made at ×400 magnification.

Leaf lesions were irregular or angular, often delimited by veins, mostly < 5 mm in diameter, sometimes up to 10 mm or more when coalesced, pale brown to grayish white in the center, and surrounded by dark purplish halo (Fig. 1A~1D). Conidiomata were pycnidial, amphigenous, but mostly epigenous, scattered, dark brown to rusty brown, globose, immersed in host tissue, becoming partly erumpent, separate, unilocular,  $50~90~(\sim110)~\mu m$  in diameter, with an ostiole of  $8~16~\mu m$  in diameter (Fig. 1C~1G). Conidia were filiform, sub-straight to mildly flexuous, truncate at the base, obtuse at the apex, hyaline, guttulate with small oil drops,  $25~42 \times 1.5~2~\mu m$ , mostly 3-septate, occasionally 1~2-septate, with indistinct septa (Fig. 1H). No teleomorphic state was observed.

Five species of *Septoria* have been described on *Centella* (= *Hydrocotyle* p. p.). They are *S. asiaticae* Speg., *S. centellae* G Winter, *S. hydrocotyles* Desm., *S. hydrocotylicola* Speg., and *S. nedodes* Kalchbr. Recently Priest [5]

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Characteristics	s Present isolate	S. centellae (Winter, 1887)	S. centellae (Bai, 2003)	S. centellae (Priest, 2006)	S. hydrocotylicola (Spegazzini, 1908)	S. hydrocotylicola (Priest, 2006)
Lesion	Center brown to gray, margin dark purplish, up to 5 mm diam.		Center yellowish brown, margin purplish brown, 1~3 mm diam.	Center mid-brown, margin purple- brown, 2~10 mm diam.	Center brown, margin purplish, 0.5~4 mm diam.	Center mid-brown, margin mid-brown, 1~5 mm diam.
Conidiomata	50~90 (~110) μm diam., mostly epigenous	Not described	60~100 μm diam., mostly epigenous	70~120 μm diam., mostly hypogenous	60~70 μm diam., s epigenous	80~110 μm diam., epigenous
Ostiole	8~16 μm wide	Not described	Not described	10~30 μm wide	Not described	25~35 μm wide
Conidia	$25\sim42\times1.5\sim2 \mu\text{m},$ (1~)3-septate	30~45 × 2 μm, multiseptate	$20\sim30\times1.5\sim2$ µm, $1\sim3$ -septate	$30\sim50\times2\sim2.5 \mu\text{m}$ , $3\sim4$ -septate	$40\sim50\times1$ µm, 3-septate	$17\sim36\times1.0\sim1.5 \mu\text{m}$ , Septa indistinct
Host species	Centella asiatica	Centella asiatica	Centella asiatica	Centella asiatica	Hydrocotylesp.	Centella asiatica, Hydrocotyle spp.

**Table 1.** Comparison of morphological characteristics of *Septoria* species known from *Centella* and *Hydrocotyle* plants

synonymized *S. asiaticae* under *S. hydrocotylicola*, and *S. nedodes* under *S. hydrocotyles*. Therefore, three species are currently accepted. Of these, *S. hydrocotyles* is very distinctive due to its falcate conidia, a characteristic mentioned in the original description and by subsequent authors [5]. The remaining two species, *S. centellae* and

S. hydrocotylicola, can be differenciated by the dimensions of their conidia and conidiomata [4, 5]. Based on the morphological characteristics of the fungus and the host genus (Table 1), the materials collected in Korea are in agreement with S. centellae G. Winter [4, 5, 8].

As shown in Table 1, however, the morphological



**Fig. 1.** A, B, Leaf spots on the leaves of *Centella asiatica* infected with *Septoria centellae*; C, Close-up view of lesions showing several conidiomata; D, Conidiomata formed on the leaf lesion; E, Conidia cirrhi formed from ostioles of conidiomata; F, Conidiomata viewed by bright-field microscope; G, Close-up view of conidiomata; H, Conidia; I, Five-week-old colonies growing on potato dextrose agar.

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differences between *S. centellae* and *S. hydrocotylicola* are not distinct enough to distinguish them from each other. These two species have been described by only a few mycologists with insufficient information (cf. Table 1). The difference between the two species might be no more than dimensions of conidiomata and conidia. Therefore, at present, it is the best choice to adopt the older name, *S. centellae*. Furthermore, this species was originally described from *Centella asiatica* [8], and *S. hydrocotylicola* was from *Hydrocotyle* sp. [9].

To solve this complexity, molecular analyses of specific genetic loci are recommended for species and genus delimitation in the genus Septoria [3, 10]. Unfortunately, there are no loci known that enable species delimitation [10]. Monoconidial isolates would be helpful and indispensable to solve this complexity. Presently, the fungus was isolated as a pure culture by aseptically removing spore cirrhi from conidiomata on the surface of leaf lesions (Fig. 1E) and placing them in a drop of sterilized water on a glass slide. A loop of conidial suspension was then streaked on the surface of the potato dextrose agar (PDA) plates using a disposable bacterial loop. Single conidial colonies were transferred to a fresh PDA plate when the conidia started to grow. The colonies formed dark grayish hyphae on PDA plates (Fig. 1I). We obtained two isolates, which have been deposited in the Korean Agricultural Culture Collection (KACC). The isolate KACC43218 was cultured from KUS-F23134 (Korea, Jeju, November 1, 2007, leg. H. D. Shin & M. J. Park) and KACC45706 from KUS-F25398 (Korea, Wando, October 4, 2010, leg. H. D. Shin, M. J. Park & J. H. Park).

There have been no previous records of *Septoria* or *Septoria*-like fungi on *C. asiatica* in Korea. To the best of our knowledge, this is the first record of this fungus as well as its host plant in Korea. The host plant has been used for medicinal purpose in Asian countries [11] and also for ground cover in Korea. Epidemic occurrence of leaf spots associated with this fungus would be a threat

for the health of this plant.

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