

## Three *Alternaria* Species Pathogenic to Sunflower

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***Alternaria helianthi* and two unreported species of *Alternaria* in Korea were isolated from lesions of *Alternaria* leaf spot disease of sunflower. The unrecorded species of *Alternaria* were identified as *A. helianthinfiens* and *A. protenta* based on the morphological characteristics of conidiophores and conidia. *A. helianthi* was the dominant species, although all the three species were associated with the disease. *A. helianthi*, *A. helianthinfiens* and *A. protenta* produced similar symptoms on detached sunflower leaves. This is the first report of *A. helianthinfiens* and *A. protenta* pathogenic on sunflower in Korea.**

**Keywords :** *Alternaria helianthi*, *A. helianthinfiens*, *A. protenta*, sunflower.

*Alternaria* leaf spot and blight of sunflower (*Helianthus annuus* L.), caused by *Alternaria helianthi* (Hansf.) Tubaki & Nishihara, has been reported from many countries where the crop is growing (Anahosur, 1978). Symptoms of the disease are dark brown, oval to circular spots with pale margin and yellow halo. Spots are found on leaves, stems, petioles, sepals and petals. In severe infections, lesions become irregular by coalescing, leading to blight and defoliation and death of the plant (Tubaki & Nishihara, 1969; Mukewar et al., 1974; Yu et al., 1989). The disease is severe in wet weather and under moist conditions.

A survey of diseases of sunflower during 1998 and 2000 growing seasons in Korea revealed that the leaf spot and blight disease of the plant was wide spread and under moist conditions was responsible for considerable damage (Fig. 1-A). *Alternaria helianthi* and two unreported species of *Alternaria* from Korea were isolated from the lesions of sunflower. The unrecorded species of *Alternaria* fitted the descriptions of *A. helianthinfiens* Simmons, Walcz & Roberts (Simmons, 1986), and *A. protenta* Simmons (Simmons, 1986; Simmons, 1997). Although *A. helianthinfiens* and *A. protenta* were reported from sunflower by Simmons (1986), their pathogenicity to the crop was not tested.

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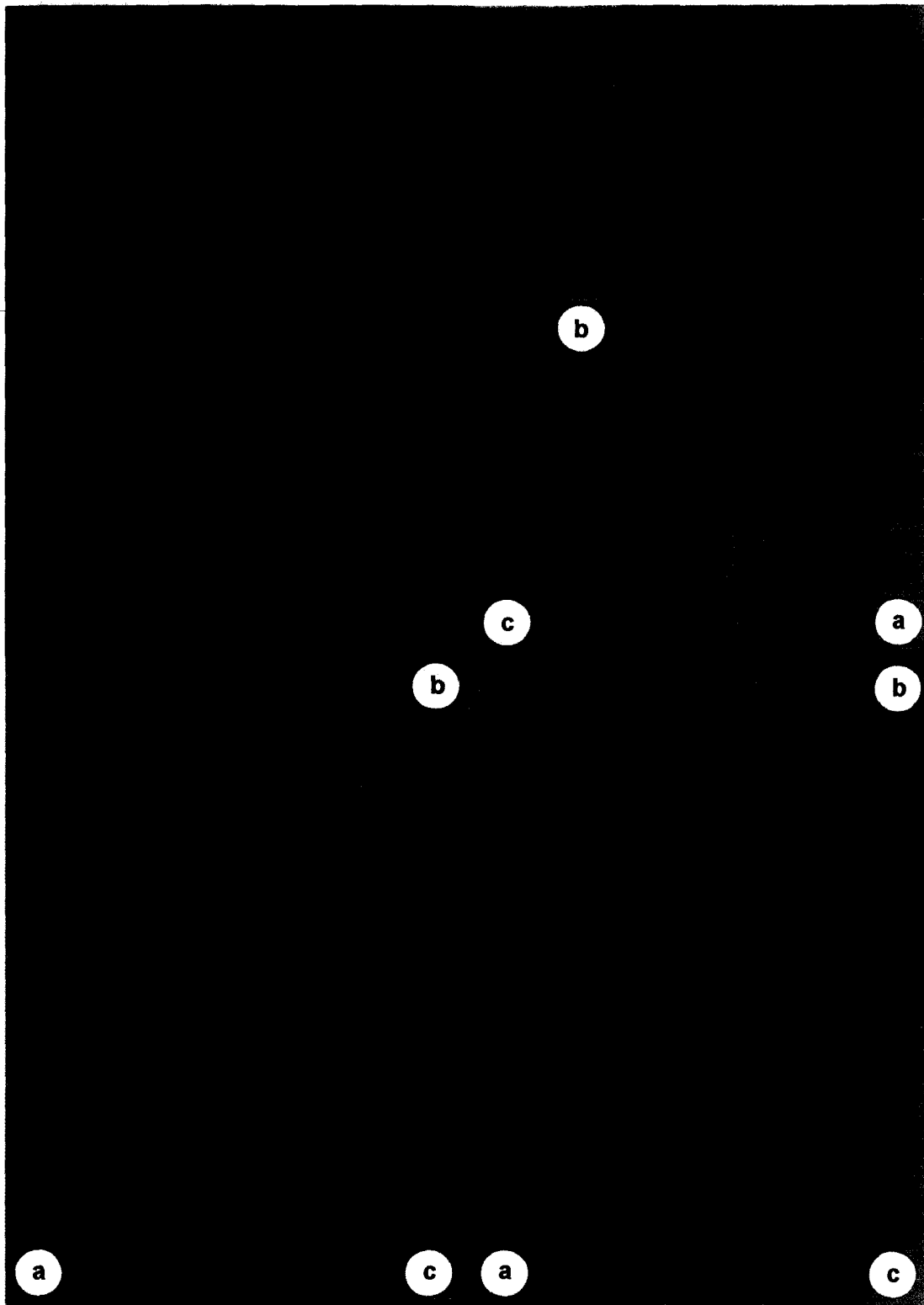
In this paper we report the three species of *Alternaria* found on sunflower, and their pathogenicity to the crop.

**Isolation of *Alternaria* spp.:** Pieces of tissue from the margins of the lesions on sunflower leaves were immersed in 1% NaOCl solution for 1 min, rinsed in sterile water and incubated on potato dextrose agar (PDA) at 25 ± 1°C. Spores were also directly transferred from lesions on leaves to PDA and incubated under the same condition. Stock cultures were prepared from the colonies by transferring mycelial plugs to PDA slants for further study.

**Identification of *Alternaria* spp.:** Microscope slides of conidiophores and conidia of the isolates were prepared from the lesions after incubation in a damp chamber for 24 h by mounting them in distilled water or lactophenol. The shape, size, surface ornamentation and number of septa of the conidiophores and conidia were recorded.

***Alternaria helianthi* (Hansf.) Tubaki & Nishihara (Fig. 1-B):** Conidiophores arising singly or in groups, simple or branched, straight or flexuous, cylindrical, pale gray to pale brown, smooth, up to 5-septate, sometimes geniculate with 1-2 scars, up to 130 µm long, 6-12 µm thick. Conidia erostate, mostly solitary, rarely produce a short apical or lateral pseudostroma that functions as conidiophore to a second conidium, straight or slightly curved, long cylindrical to slightly obclavate, rounded at both ends, subhyaline to light yellow or golden brown, smooth walled, constricted at the transverse septa, 40-140 µm long, 15-35 µm thick in the broadest part, with 2-12 transverse septa and 1 longitudinal or oblique septum (rarely 2) in each of 1-3 of the broadest divisions. Colonies on PDA light brown to olive brown, velvety, slowly growing, 15-20 mm (in darkness) or 30-35 mm (under light) in diam after 20 days at 25°C, sporulation abundant. The above characteristics fitted well to the description of *A. helianthi* (Hansf.) Tubaki & Nishihara (Tubaki & Nishihara, 1969; Mukewar et al., 1974; Anahosur, 1978; Simmons, 1986).

***Alternaria helianthinfiens* Simmons, Walcz & Roberts (Fig. 1-C):** Conidiophores arising singly or in groups, simple or branched, straight or flexuous, cylindrical, pale brown, smooth, septate, sometimes 1-geniculate, up to 150-200 µm long, 5-8 µm thick. Conidia mostly solitary, rarely in chains of 2, ellipsoid or ovoid, medium brown, smooth to minutely rough or sometimes verrucose, young conidia



**Fig. 1.** Symptoms of leaf spot disease caused by natural infection of *Alternaria* spp. on sunflower (A); Conidiophores (a), young conidia (b) and mature conidia (c) of *A. helianthi* (B), *A. helianthificiens* (C), and *A. protenta* (D) from diseased leaves. Each bar represents 50  $\mu$ m.

commonly beakless with a conoid apical cell, mature conidia have a long flexuous filiform beak, the point of

transition from spore body to beak is definite and conspicuous, with 2-8 transverse septa and 1 to 2 longitudinal septa

in 2-7 of the transverse compartments, measure 25-90 × 10-25 µm plus a beak about 100-200 µm long. Colonies on V-8 juice agar grow somewhat slowly with little mycelium and moderate amount of submerged mycelium, a yellowish red pigment was produced. Based on the above characteristics, the fungus was identified as *A. helianthinficiens* Simmons, Walcz & Roberts (Simmons, 1986).

***Alternaria protenta* Simmons (Fig. 1-D):** Conidiophores arising singly or in bundles, simple or branched, straight or slightly flexuous, more or less cylindrical, pale gray or pale brown, smooth, septate, sometimes geniculate with 1 to several scars, 50-130 µm long, 3-7 µm thick. Conidia mostly solitary, occasionally in chains of 2, long-ellipsoid, straight or slightly curved, light brown to medium brown, smooth to minutely rough or sometimes verruculose; conidium body 40-120 µm long, 12-20 µm thick in the broadest part, with 5-14 transverse septa and 1 longitudinal septum (rarely 2) in 1-7 of the transverse compartments; beak long and straight to flexuous filiform, pale or colourless, septate, often once branched, 80-150 µm long, 3-5 µm thick at the base and tapering to 2 µm tip. Colonies on PDA cottony, white to pale gray, reaching about 60 mm in diam after 7 days at 25°C. The above characteristics enabled the fungus to be identified as *Alternaria protenta* Simmons (Simmons, 1986; Simmons, 1997).

**Occurrence of *Alternaria* spp.:** A survey of *Alternaria* leaf spot disease was made in different fields during August-October of 2000. Twenty five isolations per field were made from leaf spot lesions from specimens taken from 7 of the fields. The lesions were surface-sterilized in a 1% NaOCl solution and plated on V-8 juice agar.

From 7 sunflower fields surveyed, *A. helianthi* was isolated from plants in 7 fields, and *A. helianthinficiens* and *A. protenta* from 3 fields (Table 1). The result indicates that *A. helianthi* is more prevalent than *A. helianthinficiens* and *A. protenta* in the fields, although all three species are associated with the *Alternaria* leaf spot complex in sunflower.

**Pathogenicity of *Alternaria* spp.:** For determine the pathogenicity of the three species of *Alternaria*, two isolates of each species were used. The inoculum was produced by transferring mycelial plugs from a stock culture of each isolate to fresh V-8 juice agar in plastic Petri dishes. The pure cultures were incubated at 22°C in a 12 h dark/12 h light regime conducive to sporulation for 14 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 sunflower. The detached leaves were inoculated with conidial suspension at the concentration of 2 × 10<sup>4</sup> spores/ml. Conidial suspension of each species was sprayed over the leaves with a sprayer until the

**Table 1.** Occurrence of *Alternaria helianthi*, *A. helianthinficiens* and *A. protenta* in seven sunflower fields as indicated by recovery from isolations on V-8 juice agar from surface-sterilized lesions of typical *Alternaria* leaf spot

Location of sunflower fields	<i>Alternaria</i> species recovered <sup>a</sup>		
	<i>A. helianthi</i>	<i>A. helianthinficiens</i>	<i>A. protenta</i>
Cheongyang, Chungnam	+	+	+
Gongju, Chungnam	+	-	-
Yeongi, Chungnam	+	-	-
Andong, Kyongbuk	+	+	+
Jinju, Kyongnam	+	-	-
Suwon, Kyonggi	+	-	-
Yuseong, Taejon	+	+	+

<sup>a</sup>-; absence of the species, +; presence of the species.

leaves were uniformly wet. Control leaves were sprayed with distilled water. The inoculated leaves were placed on the moistened spongy layer in the plastic boxes, incubated at 22-25°C and observed for symptom development. Each test was conducted with 3 replicates and with more than 5 leaves per replicate.

All the three species of *Alternaria* were pathogenic following inoculation on sunflower leaves. *A. helianthi* inoculated leaves showed their first symptoms about 1-2 days after inoculation. Numerous brown spots developed on leaves and prominent chlorotic halos were evident around the spots. First symptoms caused by *A. helianthinficiens* and *A. protenta* appeared about 2-3 days after inoculation. The symptoms were similar to those caused by *A. helianthi*. No visible symptom was observed on the leaves treated with distilled water.

Among the three species of *Alternaria*, *A. helianthi* was previously recorded in Korea (Yu et al., 1989), but the other two species were not known. This is the first report of *A. helianthinficiens* and *A. protenta* on sunflower in Korea and also appears to be the first record to confirm their pathogenicity to sunflower.

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