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Short Communication

Leaf Spot of *Farfugium japonicum* Caused by *Alternaria cinerariae* Intercepted at Plant Quarantine in Japan

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Abstract: A new disease of *Farfugium japonicum* (L.) Kitam. causing leaf spots was intercepted at plant quarantine inspection at Narita International Airport in April 2007. The isolated fungus was pathogenic to the original host and two Compositae plants (cineraria and silver groundsel), and identified as *Alternaria cinerariae* Hori & Enjoji based on the morphology. The result of the identification was well supported by the pathogenicity test and phylogenetic analysis of the rDNA-ITS region and the *gpd* gene fragment. This is the first report of this disease in Japan.
Key words: *Alternaria cinerariae, Farfugium japonicum*, leaf spot, plant quarantine

Farfugiun japonicum (L.) Kitam. [syn. *Ligularia tussilaginea* (Burm. F.) Makino, Compositae] is a plant native to East Asia (KITAMURA *et al*., 1994) and also recently imported into Japan as an ornamental plant. In April 2007, black and circular leaf spots were found on the plug seedlings of *F. japonicum* planted in cell trays imported from the United States at plant quarantine inspection at Narita International Airport in Japan. We aimed to diagnose the disease and identify the causal organism in this study.

Symptoms

Brown to black and circular spots 0.5 to 1 cm in diameter were observed on the leaves (Plate 1 A). Sometimes small light brown spots with a halo were produced (Plate 1 B). Lesions with the center concaved (Plate 1 C) and coalesced lesions were also observed. No sporulation was found on any lesions. Lesions were sometimes observed in leaves in contact with the diseased ones because the plants were cultivated densely in the cell tray.

Isolation of the causal fungus

Lesions on the leaves were cut into small pieces of about 5×5 mm, and the pieces were surface-sterilized for 10–20 sec with 70% ethanol, rinsed in 1% sodium hypochlorite for 2 min, then washed in sterilized distilled water. After removing excess moisture, the pieces were incubated on synthetic low nutrient agar (SNA) (NIRENBERG, 1976) plates at 25°C under darkness for 2–3 days. A hyphal tip was transferred to SNA and five isolates (IM298-A to -E) were obtained. The five isolates were used for morphological observation and two of them (IM298-A and -E) for pathogenicity tests and molecular phylogenetic analyses.

Morphological and cultural characteristics

The isolates were pre-incubated on V8 juice agar at 25°C under darkness until the colony diameter reached one-third to one-half of the 9 cm plate, whereas the growing rate was about 0.7 cm/day. Aerial hyphae were removed from the surface of the colony with a spatula to induce sporulation. Subsequently, the plates were placed under irradiation by black light blue (BLB) with alternating 12 h/light (25°C) and 12 h/darkness (20°C) for 2–3 days. As a result, conidia were formed mainly at the first cycle of irradiation, and a concentric ring was produced in the colony (Plate 1 D). In slide culture, pieces of agar including hyphae were cut after removing aerial hyphae and incubated between a slide glass and a cover glass under the same condition. The morphological characteristics (Plate 1 E, F) were similar among the isolates, as shown in Table 1. Conidiophores are singly, simple or slightly curved, up to 180×7–8 μm, pale to mid brown,

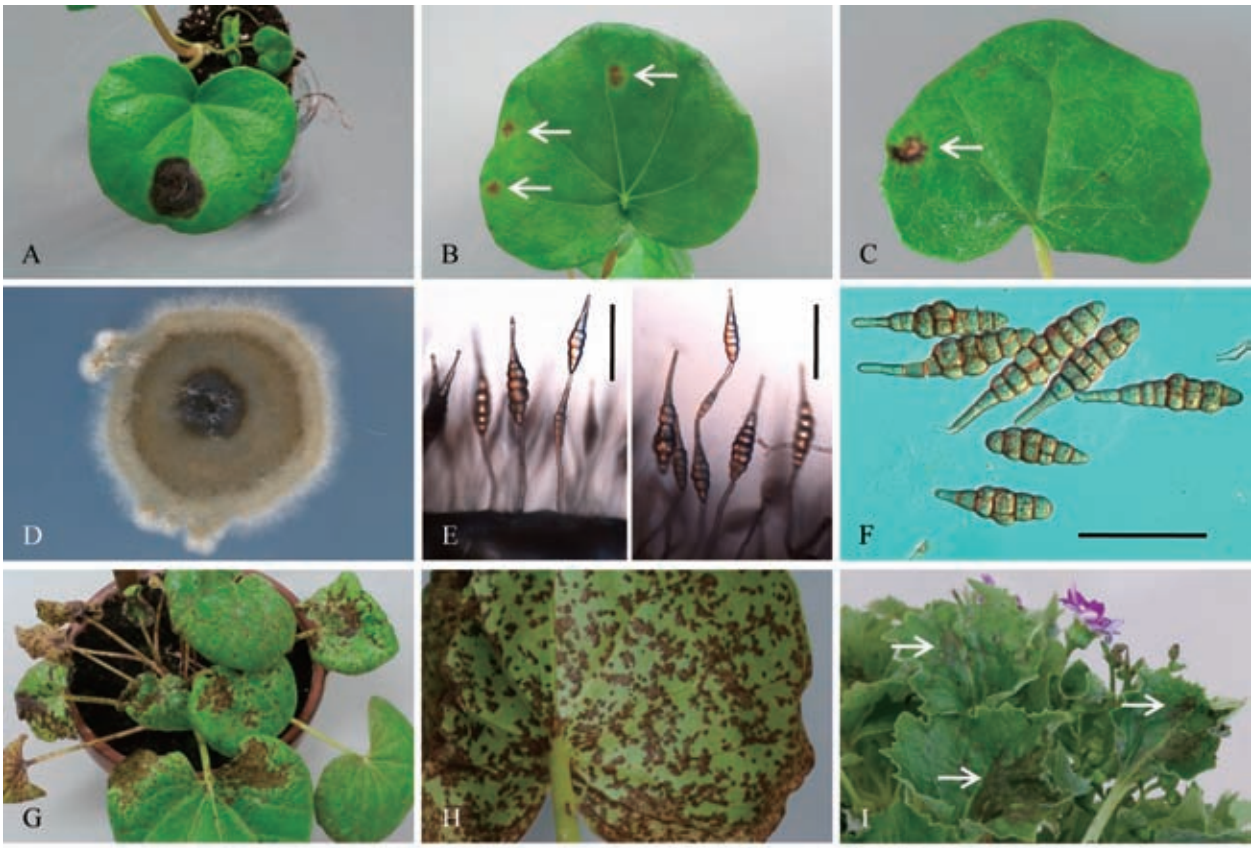

Plate 1. A–C: Symptoms on *F. japonicum* intercepted at plant quarantine in April 2007 (A: Black and circular lesion on a leaf, B: Small and brown lesions with halo, C: A concaved lesion). D: Colony of the isolate (No. IM298-A) incubated on V8 juice agar for 3 days under alternating 12 h/black light blue (25°C) and 12 h/darkness (20°C). E: Conidiophore and conidia produced solitarily or in 2 (left) and 3 chains (right) on slide culture. Bar 100 μm. F: Conidia of the isolate (No. IM298-A) Bar 100 μm. G–J: Symptoms on three Compositae plants 2–3 days after inoculation with conidial suspension without wounding (G: *F. japonicum*, H: Undersurface of G, I: Cineraria [*Senecio cruentus*], and J: Silver groundsel [*S. cineraria*]).

and bear conidia at the apex. Conidia are mostly solitary, rarely in chains on agar plates, sometimes in 2–3 chains on slide cultures (Plate 1 E), obpyriform or obclavate, golden brown to brown, with 3–9 (avg. 5.8) transverse and 0–6 (avg. 2.6) longitudinal septa, constricted at the septa, body 50–132.5×17.5–37.5 μm (avg. 87.5×28.7), with a broadly tapered or short conical beak, up to 62 μm long, 5–16.3 μm wide (Plate 1 F).

Pathogenicity test

The potted original host (*F. japonicum*) and two kinds of Compositae plants, cineraria (*Senecio cruentus* DC.) and silver groundsel (=dusty miller, *S. cineraria* DC.), which were previously reported as the host of *A. cinerariae* in Japan, were used for the experiment. Two or three for each plant were prepared after cultivating healthy and symptomless plants in a green house for more than a week. Inoculation was done by spraying conidial suspensions of 10^3–10^4 conidia/ml on the plants without wounding. Sterilized distilled water was sprayed as a control. The inoculated plants were held in a plastic case with a cover to keep humidity for 1–2 days, then the cover was removed and the plants were kept in a growth chamber at 22°C under alternating 12 h/fluorescent light and 12 h/darkness. Three to four days after inoculation, similar symptoms were reproduced on the original host. Numerous lesions of 1–2 mm in diameter on leaves, petioles, or stems and severe blight at the leaf margin appeared on the original host (Plate 1 G, H). Leaf blight appeared on cineraria (Plate 1 I) and small brown spots only on lower leaves of silver groundsel (Plate 1 J). Inoculated fungi were readily reisolated from lesions of each plant. The isolates were demonstrated to be pathogenic to these three kinds of plants.

Table 1 Comparison of conidial characteristics and host plants of isolates causing leaf spot of *Farfugium japonicum* with those of *A. cinerariae* described previously.

	Isolates (n=5)	*A. cinerariae* previously described by			
		ENJOJI (1931)	ELLIS (1976)	TAKANO (2001)	SIMMONS (2007)
Substrata	V8 juice agar	on symptom	–[*1]	on symptom	V8 juice agar?
Sporulation	solitary or 2–3 chains	in chains	often in short chains	in short chains	predominantly solitary or a few chains
Total					
Shape	obpyriform to obclavate	long or short obclavate	obpyriform to obclavate, rostrate	long or short obclavate	broadly ellipsoid or ovoid, or obclavate
Length	72.5–170 μm (avg. 121.9)	77.5–177.5 (–240) μm[*3] (avg. 124.8)	–	135–191 μm	160–230 μm[*4]
Body					
Length	50–132.5 μm (avg. 87.5)	–	50–140 μm	95–118 μm	–
Width	17.5–37.5 μm (avg. 28.7)	12.5–25.0 μm (avg. 17.6)	15–40 μm	32–46 μm	32–42 μm
No. of septa					
Transverse	3–9 (avg. 5.8)	5–11 (avg. 8.05)	3–10	5–11	8–11
Longitudinal	0–6 (avg. 2.6)	0–3	several	several	1–2
Color	golden brown to brown	pale yellowish green, yellowish brown, or brown	golden brown	pale yellow to yellowish brown	medium yellow tan
Constriction at the septa	pronounced	–	pronounced	pronounced	pronounced
Beak					
Shape	broadly tapered or conical	narrow long taper	–	–	rounded or short, broadly tapered
Length	up to 62 μm	–	up to 80 μm	28–81 μm	–
Width	5–16.3 μm	–	6–9 μm	7–12 μm	–
Hosts	*Farfugiun japonicum* *Senecio cruentus*[*2] *S. cineraria*[*2]	*Senecio cruentus*	*Senecio cruentus*	*Senecio cineraria* *S. cruentus*[*2]	*Senecio cruentus* *S. cineraria* *S. skirrhodon* *Ligularia* sp. *Pericalis* sp.

*1. –: Not described.
*2. Based on inoculation tests.
*3. ENJOJI (1931) described conidial length to become longer (up to 240 μm long) in humid conditions.
*4. SIMMONS (2007) measured only matured conidia.

Phylogenetic analysis

The sequence of the internal transcribed spacer of ribosomal DNA (rDNA-ITS) including 5.8S, ITS1, and ITS2 regions and the glyceraldehyde-3-phosphate dehydrogenase gene fragment (*gpd*) were analyzed to confirm the species identification. These regions were amplified with the primers ITS1/4 (WHITE *et al.*, 1990) and *gpd*1/2 (BERBEE *et al.*, 1999), respectively, directly sequenced, and then compared with those of other *Alternaria* species registered in the DNA Data Bank of Japan (DDBJ). Both the isolates tested (IM298-A and -E) had 100% homology in the sequences of rDNA-ITS and *gpd*. The rDNA-ITS region of the fungus had 99.8% (481/482 bp) similarity with *A. cinerariae* (DDBJ accession No. AY154700). HONG *et al.* (2005) reported that *A. cinerariae* composed a monophyletic group with *A. sonchi* (sonchi-species group), and the two species can be distinctly distinguished based on the sequence of *gpd*. The neighbor-joining tree based on the sequence of *gpd* showed that the isolates fell into the clade of *A. cinerariae* and were clearly separated from the other *Alternaria* species (Fig. 1). The sequences of *gpd* of the isolates had 99.8% (555/556 bp) homology with *A. cinerariae* (AY562413) and 98.4% (547/556 bp) with *A. sonchi* (AY562412).

Identification

Based on the morphology of conidiophores and conidia, all isolates were considered to belong to the genus *Alter-*

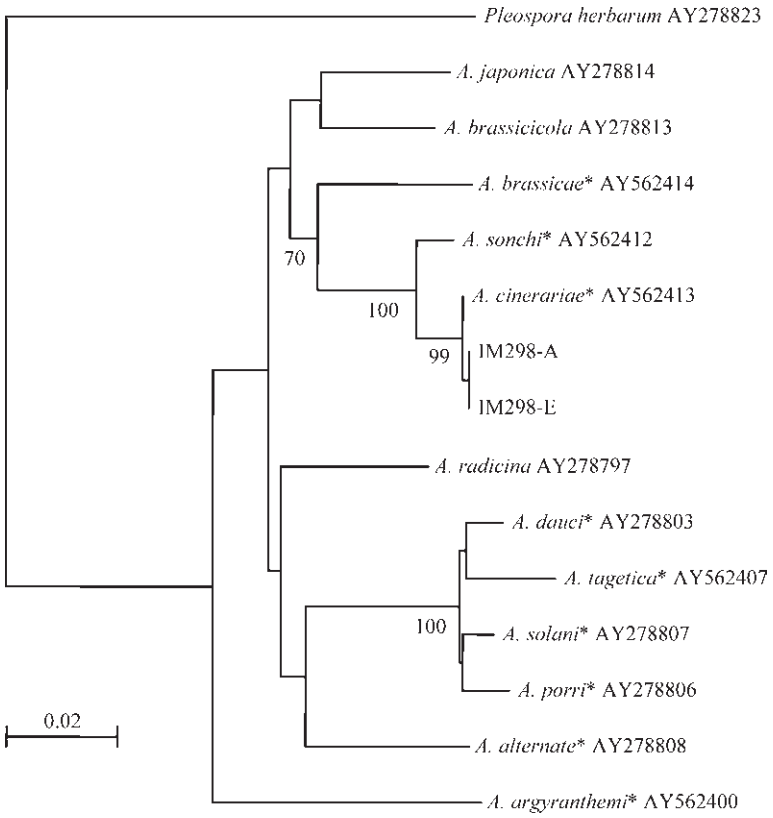

Fig. 1. A phylogenetic tree of *Alternaria* species, including our isolates based on *gpd* sequences by neighbor-joining analysis. Numbers under the branches are bootstrap values in 1000 bootstrap replicates. The bootstrap values greater than 70 are shown. The accession numbers in the DNA Data Bank of Japan (DDBJ) are shown to the right of the species name.
*: *Alternaria* species whose host plants belong to Compositae.

naria. The isolates (IM298-A to -E) were compared with the previous descriptions of *Alternaria* species, and similar to the morphology of *A. cinerariae* (Table 1) and *A. sonchi*. The morphological characteristics of isolates almost agreed with those of *A. cinerariae*, especially with the total shape of conidia and broadly tapered conidial beak, although there were some differences in conidial sizes compared with ENJOJI (1931) and SIMMONS (2007), which were considered to be within variation depending on the maturity or culture conditions. On the other hand, *A. sonchi* could be distinguished from isolates based on the conidial size, which were 72.5–170 μm in the isolates and 50–100 μm in *A. sonchi* (SIMMONS, 2007). In results of the pathogenicity test, the isolates (IM298-A and -E) were pathogenic to both cineraria and silver groundsel, which were previously reported as host plants of *A. cinerariae* (ENJOJI, 1931; TAKANO, 2001). In conclusion, all isolates were identified as *Alternaria cinerariae* Hori & Enjoji, based on the morphology. The result of identification was well supported by the pathogenicity test and phylogenetic analysis of rDNA-ITS and *gpd*. *A. cinerariae* was reported as a serious pathogen of cineraria in Japan (ENJOJI, 1931). Until now, this fungus has been known to infect mainly *Senecio* spp. (Compositae) in Japan, Korea, the United States, Denmark, England, and New Zealand (FARR *et al*., 2009), but not reported from *F. japonicum* (syn. *L. tussilaginea*) in Japan (THE PHYTOPATHOLOGICAL SOCIETY OF JAPAN, 2000) or even in the United States (DAVID *et al*., 1989), where the imported plants tested in this study were grown. This is the first report of *A. cinerariae* causing leaf spot disease in *F. japonicum* in the world, although *Ligularia,* a closely related genus to *F. japonicum*, was listed as a host of the fungus (SIMMONS, 2007). We propose the disease of *F. japonicum* as leaf spot (Japanese name: hanten-byo) caused by *A. cinerariae*. The symptoms of this disease are similar to those of black leaf spot caused by *Phoma* sp. that produces no pycnidia as a sign on the lesions (FURUKAWA and KISHI, 2004). Therefore, it is necessary to isolate the causal fungus to distinguish leaf spot from black leaf spot.

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和 文 摘 要

輸入検疫で発見されたツワブキ斑点病（新称）

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2007年4月、成田国際空港における輸入検疫でアメリカ合衆国産ツワブキ苗の葉に褐色～黒色、円形の斑点が認められ、*Alternaria*属菌が高率に分離された。分離菌は無傷噴霧接種でツワブキに原病徴を再現し、サイネリアおよびシロタエギクにも病原性を示した。いずれの植物からも接種菌が再分離された。本菌の分生子は単生あるいは2–3連鎖し、黄褐色～褐色、倒洋梨形～倒棍棒形で、横隔壁部がくびれる。本体部は50–132.5 × 17.5–37.5 μm、横隔壁数3–9、縦隔壁数0–6、嘴部は最長62 μm、幅5–16.3 μmである。形態的特徴から本菌を*A. cinerariae* Hori & Enjojiと同定した。同定結果は、接種試験およびrDNA-ITSと*gpd*遺伝子領域の分子系統解析から支持された。本菌によるツワブキの病害は我が国未報告のため、斑点病（英名：Leaf spot）と呼称することを提案する。