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Wilt of Perilla Caused by *Fusarium* spp.

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A survey of Fusarium wilt of perilla was conducted in 12 locations in Korea from 1999 to 2001. The disease occurred in 74 out of 187 fields in the 12 locations surveyed, and incidence of the disease reached up to 30% at its maximum in some perilla fields in Seosan and Dangjin. Incidence of the disease in the other locations ranged from 0.2 to 20%. A total of 327 isolates of *Fusarium* spp. were obtained from stems and roots of the diseased perilla plants. The isolates were identified based on their morphological characteristics. Out of the 327 isolates of *Fusarium*, 277 isolates from 12 locations were identified as *F. oxysporum*, 11 isolates from three locations as *F. solani*, 17 isolates from two locations as *F. equiseti*, 4 isolates from one location as *F. avenaceum*, and 6 isolates from one location as *F. subglutinans*. The other 12 isolates of *Fusarium* from four locations were unidentified. Twelve isolates of *F. oxysporum* and two isolates each of the other *Fusarium* spp. were tested for their pathogenicity to five cultivars of perilla. Seven isolates of *F. oxysporum* were strongly pathogenic to some perilla cultivars, but the other five isolates were weakly or not pathogenic. One isolate of *F. solani* was strongly pathogenic to all the perilla cultivars tested, but another isolate was not pathogenic. All the isolates of *F. equiseti*, *F. avenaceum*, and *F. subglutinans* tested were not pathogenic to any of the perilla cultivars tested. Symptoms on the perilla plants induced by artificial inoculation with strongly pathogenic isolates of *F. oxysporum* and *F. solani* appeared as wilt, stem blight, and root rot, which were similar to those observed in the fields. The isolates which induced symptoms by artificial inoculation were re-isolated from the lesions of the perilla plants inoculated. All the isolates of *F. oxysporum* tested were not pathogenic to eight other crops inoculated. Results of this study reveal that *F. oxysporum* is the main pathogen of perilla wilt and that it is host specific to perilla. A *forma specialis* of *F. oxysporum* causing wilt of perilla is proposed as *perillae*.

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Perilla [*Perilla frutescens* (L.) Britton var. *japonica* (Hassk.) Hara] is grown as an oil crop in east Asian countries. The plant is also grown as a vegetable crop under greenhouse conditions in Korea. Growers usually cultivate perilla in open fields to harvest seeds for oil production during summer. During a disease survey of perilla in several locations in Korea, severe outbreaks of wilted plants were sometimes observed. The diseased plants showed rot symptoms on basal parts of stems and roots. *Fusarium* spp. was consistently isolated from the diseased plant parts, which suggested that the fungi are associated with the disease.

It has been reported that *Fusarium* spp. is widely distributed in the soil and mostly cause wilt on a variety of plants (Armstrong and Armstrong, 1975; Booth, 1971; Snyder and Hansen, 1940). However, there have been no reports on wilt of perilla caused by *Fusarium* spp. except a listed record on stem rot of perilla caused by an unknown species of *Fusarium* (Anonymous, 1998). This study was conducted to investigate the occurrence of perilla wilt in Korea and to determine the etiological characteristics of the causal *Fusarium* spp.

Materials and Methods

Survey and collection of diseased plants. Perilla fields in 12 locations in Korea were surveyed in July through October from 1999 to 2001. Perilla plants with wilt symptoms were collected, and the lesions were anatomically examined.

Isolation of pathogens. Lesion pieces (3-5 mm) cut from the diseased plants of perilla were plated on 2% water agar medium (WA) after surface-sterilizing with 1% sodium hypochlorite solution for 1 minute. Fungal isolates obtained from the lesion pieces were transferred to potato dextrose agar (PDA) slants. Isolates of *Fusarium* spp. identified by morphological observation under a light microscope were cultured for sporulation on PDA at 25% for 10-20 days. Using an inoculating needle, conidia

produced on PDA were suspended in 200 μl of sterile distilled water in a 1.5 ml microtube to make a conidial suspension. A loopful of the conidial suspension was streaked on the WA surface with a platinum wire loop to distribute the conidia. After 12-20 h incubation at 25%, agar fragments bearing a single germinated conidium were transferred to fresh WA and incubated at 25% for 5 days. Single-conidium isolates obtained from the WA plates were cultured on PDA and used for the identification and pathogenicity tests.

Investigation of morphological characteristics. Each isolate was cultured on carnation leaf agar (Fisher et al., 1982) at 27% for 20-30 days in alternating cycles of 12 h NUV light and 12 h darkness. The morphological features of each culture on the medium were examined by light microscope. Fifty conidia, conidiophores, and chlamydospores chosen randomly from each culture were observed and measured under the light microscope.

Pathogenicity test. Seeds of five cultivars of perilla and two cultivars each of eight other crops were sown in circular plastic pots (21 cm in diameter and 29 cm in height) containing sterile soil, and the pots were placed in a greenhouse at 18-32%. Twenty-three- to 25-day-old plants of perilla and other crops were used for the pathogenicity tests. Twelve isolates of *F. oxysporum* and two isolates each of the other *Fusarium* spp. were tested for their pathogenicity to the five cultivars of perilla. The 12 isolates of *F. oxysporum* were also tested for their pathogenicity to two cultivars each of eight other crops.

Each isolate of *Fusarium* species was cultured on cornmeal-sand medium (23 g cornmeal: 210 g sand: 40 ml distilled water) in 500 ml-flasks for 30-40 days to prepare the inoculum. For the inoculation tests, surface soil around the plant was dug at a depth of 2-3 cm, and 50 g of each inoculum was placed on the roots. The inoculated plant parts were covered with the original soil. The same quantity of cornmeal-sand medium was used for the control plant. The inoculated and control plants were cultivated in the greenhouse at 18-32°C. The inoculation experiment was performed in three replicates. Symptoms were observed during cultivation of the inoculated plants, and disease rating was made based on the severity of wilting and rotting induced on the plants 30 days after inoculation. Re-isolation of the pathogen from the lesions on the plants was conducted as described previously.

Table 1. Occurrence of Fusarium wilt of perilla in 12 locations in Korea from 1999 to 2001

Location	No. of fields surveyed	No. of fields infected	% infected plants[a]
Cheongyang	20	8	0.2-10.0
Dangjin	20	7	0.7-30.0
Geumsan	21	9	0.2-5.0
Hongcheon	14	4	0.2-15.0
Hwacheon	12	3	0.2-5.0
Inje	11	2	0.3-5.0
Namwon	13	6	0.2-10.0
Sancheong	11	7	0.3-20.0
Seosan	22	8	0.6-30.0
Suwon	13	9	0.3-10.0
Yangpyeong	11	7	0.2-10.0
Yeoju	19	4	0.2-5.0
Total	187	74	0.2-30.0

[a] One hundred plants in each field were investigated in three to five replicates.

Results

Disease incidence and symptoms. Wilt of perilla occurred in 74 out of 187 fields in 12 locations surveyed in Korea, and the disease incidence reached up to 30% at its maximum in some perilla fields in Seosan and Dangjin (Table 1). The disease incidence in the other locations ranged from 0.2 to 20%. Symptoms appeared as wilt after the mid growing stage of perilla in the fields (Fig. 1). Diseased plants were somewhat retarded in their growth in

Table 2. Morphological characteristics of *Fusarium* spp. isolates from perilla plants[a]

Species	Shape and size (μm) of conidiophores	Shape and size (μm) of		Shape and diameter (μm) of chlamydospores
		Microconidia	Macroconidia	
Fusarium oxysporum	Monophialide, 5-20×1.5-2.5	0-2 septate, 5-29×2-5	3-7 septate, 24-65×2.5-5.0	Globose to ellipsoidal, singly or in pairs, 5-13
F. solani	Monophialide, 50-110×2.5-3.0	0-2 septate, 5-25×2-5	3-5 septate, 25-44×4-6	Globose to oval, singly or in pairs, 5-10
F. equiseti	Monophialide, 2.5-17.5×1.5-2.5	0-1 septate, 8-18×2.5-3.8	5-7 septate, 38-58×2.5-4.5	Globose, singly or in chains, 7-13
F. avenaceum	Monophialide, 6.3-20×2.0-2.5	0-2 septate, 5-13×2-3	3-7 septate, 33-70×2.5-4.5	–[b]
F. subglutinans	Monophialide, polyphialide, 5-45×2.0-2.5	0-2 septate, 5-26×2.0-3.8	3-5 septate, 25-48×2.5-3.8	–

[a] Measurement was made after incubation for 15-20 days on CLA at 27%.
[b] – : absent or rarely produced.

Fig. 1. Symptoms of wilt on perilla plants grown in the fields. (**A** and **B**) Wilted plants at the developing or late stage of the disease, respectively; (**C** and **D**) Rot of basal parts of stems and roots with black lesions; (**E**) A longitudinal section of the diseased stem showing discoloration.

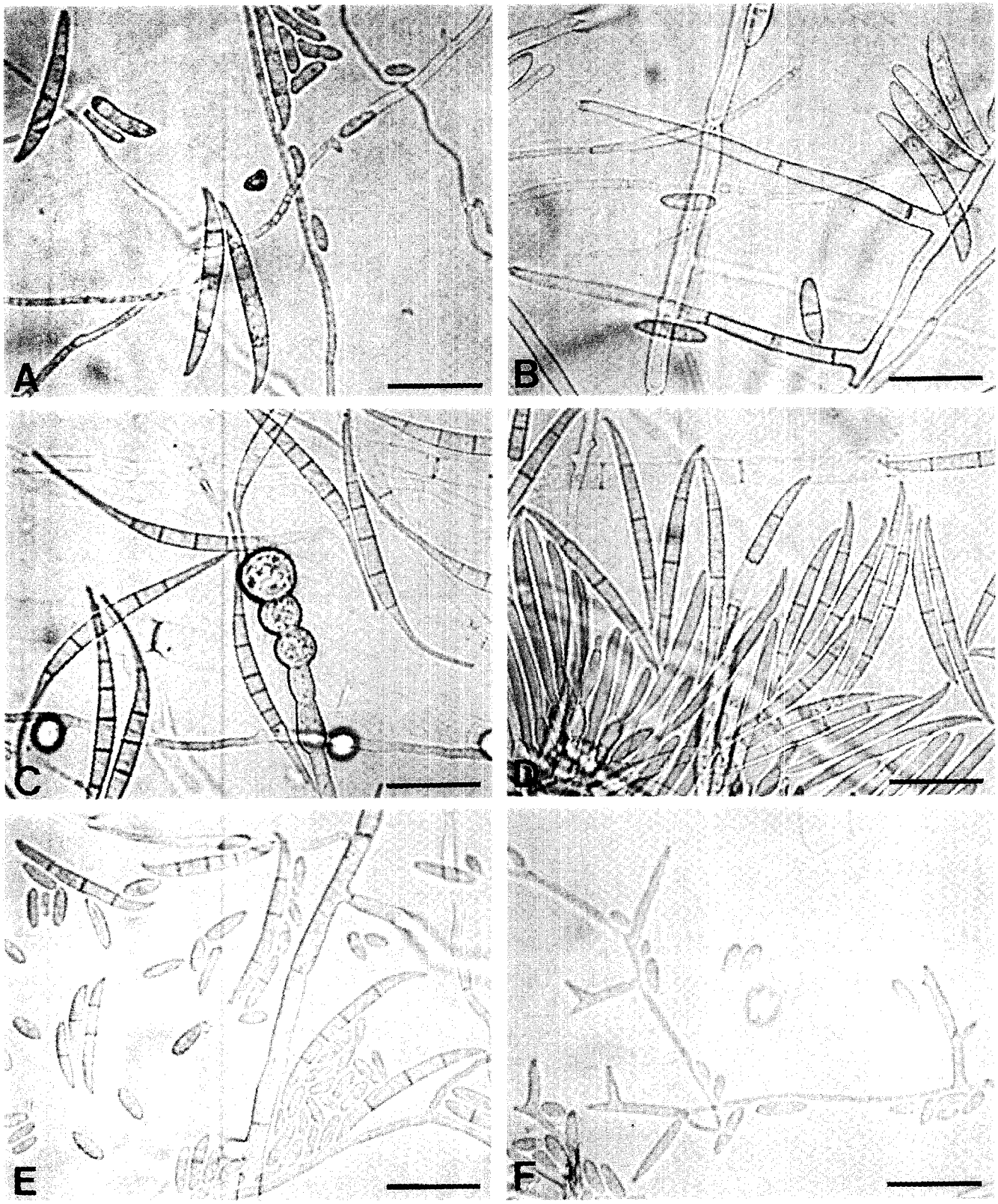

Fig. 2. Morphological features of *Fusarium* spp. isolated from perilla plants. (**A**) Microconidia, macroconidia, short monophialides, and hyphae of *F. oxysporum*; (**B**) Microconidia, macroconidia, long monophialides, and hyphae of *F. solani*; (**C**) Macroconidia and chlamydospores in a chain and hyphae of *F. equiseti*; (**D**) Macroconidia, monophialides, and hyphae of *F. avenaceum*; (**E** and **F**) Microconidia, macroconidia, mono- and polyphialides, and hyphae of *F. subglutinans*. Each scale bar = 20 μm.

Table 3. Isolation and identification of *Fusarium* spp. from perilla plants showing wilt in the fields

Species	Location	No. of isolates
Fusarium oxysporum	Cheongyang	22
	Dangjin	27
	Geumsan	16
	Hongcheon	38
	Hwacheon	9
	Inje	17
	Namwon	23
	Sancheong	15
	Seosan	38
	Suwon	10
	Yangpyeong	46
	Yeoju	16
F. solani	Hwacheon	1
	Sancheong	5
	Suwon	5
F. equiseti	Cheongyang	13
	Seosan	4
F. avenaceum	Hwacheon	4
F. subglutinans	Cheongyang	6
Fusarium sp.	Geumsan	1
	Sancheong	3
	Yangpyeong	4
	Yeoju	4
Total		327

comparison with healthy plants. Leaves of diseased plants generally showed discoloration or yellowing with wilt during the mid to late stage of the disease development, then blighted thereafter. Basal parts of stems and roots of diseased plants turned dark-brown to black and rotted in dry condition. The inside parts of the stems turned brown to dark-brown. Severely diseased plants entirely rotted and blighted.

Isolation and identification. A total of 327 isolates of *Fusarium* spp. were obtained from stems and roots of the diseased perilla plants. The isolates were identified based on their morphological characteristics (Table 2 and Fig. 2). The morphological characteristics of five *Fusarium* spp. examined by the authors were consistent with those described by previous workers (Booth, 1971; Nelson et al., 1983).

Out of the 327 isolates of *Fusarium*, 277 isolates obtained from 12 locations were identified as *F. oxysporum* Schlecht.:Fr., 11 isolates from three locations as *F. solani* (Mart.) Sacc., 17 isolates from two locations as *F. equiseti* (Corda) Sacc., 4 isolates from one location as *F. avenaceum* (Fr.) Sacc., and 6 isolates from one location as *F. subglutinans* (Wollenw and Reinking) (Nelson et al., 1983) (Table 3). The other 12 isolates of *Fusarium* from four locations were unidentified.

Pathogenicity. Out of the *F. oxysporum* isolates tested, seven isolates were strongly pathogenic to some perilla cultivars, but the other five isolates were weakly or not pathogenic (Table 4). One isolate of *F. solani* was strongly pathogenic to all the perilla cultivars tested, but another one was not pathogenic. All the isolates of *F. equiseti, F. avenaceum,* and *F. subglutinans* tested were not pathogenic to the perilla cultivars. Symptoms on the perilla plants induced by artificial inoculation with strongly pathogenic isolates of *F. oxysporum* and *F. solani* appeared as wilt and blight with stem and root rot, which were similar to those observed in the fields. The isolates which induced the symptoms by artificial inoculation were re-isolated from the lesions on the perilla plants inoculated. All the isolates of *F. oxysporum* tested were not pathogenic to eight other crops inoculated (Table 5).

Discussion

This study is the first report on wilt of perilla caused by *Fusarium* spp. Five *Fusarium* spp. were isolated from the diseased perilla plants. Among the *Fusarium* spp., *F. oxysporum* and *F. solani* induced wilt symptoms on perilla plants by artificial inoculation, suggesting that the two *Fusarium* spp. are associated with the disease occurrence in the fields. *F. oxysporum* was the most frequently isolated from the diseased perilla plants, while isolation frequency of *F. solani* and the other *Fusarium* spp. from the same host plants was very low. This suggests that *F. oxysporum* is the main pathogen of the disease.

Fusarium oxysporum has a wide host range, and many *formae speciales* of the fungus have been differentiated based on the pathogenicity of the isolates (Armstrong and Armstrong, 1975; Armstrong and Armstrong, 1981; Booth, 1971; Corell, 1991; Kistler, 1997; Snyder and Hansen, 1940). The pattern of diversity for some *formae speciales* is consistent with a monophyletic clonal population structure (Kistler, 2001). The present study showed that the *F. oxysporum* isolates from diseased perilla plants are host specific to perilla. Consequently, a *forma specialis* of *F. oxysporum* causing wilt of perilla is proposed as *perillae,* although pathogenicity tests to other plant species are needed to confirm the host specificity.

It has been reported that several races exist within the isolates of a *forma specialis* of *F. oxysporum* (Armstrong and Armstrong, 1975; Armstrong and Armstrong, 1981; Kistler, 1997; Stall and Walter, 1965). It was also reported that there are nonpathogenic strains in *F. oxysporum* isolates from plants and soil (Appel and Gordon, 1994; Booth, 1971; Snyder and Hansen, 1940). The present study

Table 4. Pathogenicity of *Fusarium* spp. isolates on five cultivars of perilla by artificial inoculation

Species	Isolate No.	Locality of isolates	Reaction of inoculation tests on cultivars				
			Areum	Jinmi	Manchu	Saeyeobsil	Yeobsil
Fusarium oxysporum	F6481	Hwacheon	+[a]	−	−	−	−
	F6491	Hongcheon	−	−	+	−	−
	F6545	Inje	+	++	++	−	−
	F6578	Yangpyeong	+	−	+	+	+
	F6615	Yeoju	−	+	+	+	+
	F6641	Dangjin	+	+	+	+	+
	F6679	Seosan	+	+	++	+	++
	F6722	Cheongyang	++	+	++	++	++
	F6763	Geumsan	+	++	+	+	++
	F6793	Sancheong	+	+	++	++	++
	F6814	Namwon	+	+	+	+	++
	F6852	Suwon	+	+	+	++	++
F. solani	F6478	Hwacheon	−	−	−	−	−
	F6796	Sancheong	++	++	++	++	++
F. equiseti	F6703	Seosan	−	−	−	−	−
	F6726	Cheongyang	−	−	−	−	−
F. avenaceum	F6471	Hwacheon	−	−	−	−	−
	F6474	Hwacheon	−	−	−	−	−
F. subglutinans	F6757	Cheongyang	−	−	−	−	−
	F6761	Cheongyang	−	−	−	−	−
Control			−	−	−	−	−

[a] ++ = wilted with severe rot of stems and roots; + = not wilted with weak rot of stems and roots; − = no symptom.

Table 5. Pathogenicity of *Fusarium oxysporum* isolates on eight crops by artificial inoculation

Isolate No.	Reaction of inoculation tests on cultivars of crops															
	Ca[a]		Cu		Ome		Ra		Se		Sp		To		Wa	
	Gu[b]	Gr	Ba	Eu	Kn	Ks	Ha	Ja	An	Ya	Gu	Pa	Se	Gw	Bo	Da
F6481	−[c]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6491	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6545	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6578	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6615	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6641	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6679	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6722	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6763	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6793	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6814	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F6852	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Control	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] Ca=cabbage; Cu=cucumber; Ome=oriental melon; Ra=radish; Se=sesame; Sp=spinach; To=tomato; Wa=watermelon.
[b] Gu=Gusto; Gr=Green Hero; Ba=Baekrokdadagi; Eu=Eunseongbaekdadagi; Kn=Keumnodajieuncheon; Ks=Keumssragieuncheon; Ha=Hanoldaehyeong; Ja=Jangchundaehyung; An=Ansan; Ya=Yangbaek; Gu=Guibin; Pa=Paroogu; Se=Seogwang; Gw=Gwangmyeong; Bo=Bok-soobak; Da=Dalsoobak.
[c] − = no symptom.

revealed that there are differences in pathogenicity among the *F. oxysporum* isolates from diseased perilla plants, and that some isolates are not pathogenic to some cultivars of perilla. The results suggest that virulence of the pathogenic

isolates of *F. oxysporum* differs depending on the perilla cultivars. Further study is needed to classify the races of the pathogenic isolates of *F. oxysporum*.

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