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Short Communication

Bulb Rot of *Sandersonia aurantiaca* Caused by *Fusarium anguioides* and *Fusarium* sp. Intercepted at Plant Quarantine in Japan

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Abstract: A new disease of *Sandersonia aurantiaca* Hook. causing bulb rot was intercepted at plant quarantine inspection at Narita International Airport in July 2005, June 2006, and August 2007. The isolated fungi were pathogenic to the original host and were identified as *F. anguioides* Sherb. and *Fusarium* sp. (within the *Fusarium avenaceum* species complex), based on the morphology, cultural characteristics, and phylogenetic analyses of the partial β-tubulin gene and TEF-1α gene. This is the first report of this disease in Japan.
Key words: *Fusarium anguioides*, *Sandersonia aurantiaca*, plant quarantine

Christmas bells (*Sandersonia aurantiaca* Hook., Liliaceae) are a bulbous plant native to South Africa (KIMURA, 1994) that has recently been imported into Japan from New Zealand as bulbs. In July 2005, June 2006, and August 2007, brown to dark brown discolored bulbs of *S. aurantiaca* were found in plant quarantine inspection at Narita International Airport in Japan. In this study, we aimed to diagnose the disease and identify the causal organisms. Preliminary results have been reported elsewhere (SAKODA *et al*., 2010).

Symptoms

Brown to dark brown discoloration was initially observed at the base of the bulb (Fig. 1 A), then some lesions enlarged a little and discolored to black (Fig. 1 B). The bulbs shrank with corrugation at the diseased area (Fig. 1 B), and the section of the lesions were sponge-like appearance (Fig. 1 C). Sometimes the lesion appeared at the whole base (Fig. 1 D) or part of the base as small circular necroses (Fig. 1 E). Pale brown lesion was also produced near the base (Fig. 1 F).

Isolation of the causal fungus

Lesions in the bulbs were cut into small block pieces (ca. 3×3×3 mm), and the blocks were surface-sterilized for 20 sec with 70% ethanol, rinsed in 1% sodium hypochlorite for 2 min, then washed in sterilized distilled water. After removing excess moisture, the blocks were incubated on synthetic low nutrient agar (SNA; 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_20$, 0.5 g KCl, 0.2 g dextrose, 0.2 g sucrose, 23 g agar in 1 l of distilled water) plates at 25°C under darkness for five days. A single spore was isolated from the colony and transferred to SNA. Six isolates (No. IM100-1, IM100-2, IM210-A, IM210-B, IM367-1, and IM369-1) were obtained and used for cultural and morphological observation and pathogenicity tests, and four of them (No. IM100-1, IM100-2, IM367-1, and IM369-1) for molecular phylogenetic analyses.

Cultural characteristics

Cultural characteristics of isolates were examined on potato dextrose agar (PDA; Difco, Detroit) under darkness at temperatures ranging from 5°C to 35°C in 5°C intervals for 10 days. The surface of the colony was white and cottony with aerial mycelia in all isolates (Fig. 1 G), which were divided into two types based on the colony color in reverse (Fig. 1 H), i.e. yellow-type (No. IM100-2-Y, IM210-A-Y, IM210-B-Y, and IM369-1-Y) and red-type (No. IM100-1-R and IM367-1-R). Isolates of both types grew between 5°C and 30°C with an optimum temperature for mycelial growth at 20°C; mean diameter in 10 days was 7.8–8.1 cm in the yellow-type isolates and 9.2–9.4 cm in the red-type isolates, but with similar values (4.5–5.2 cm) at 25°C (Fig. 2).

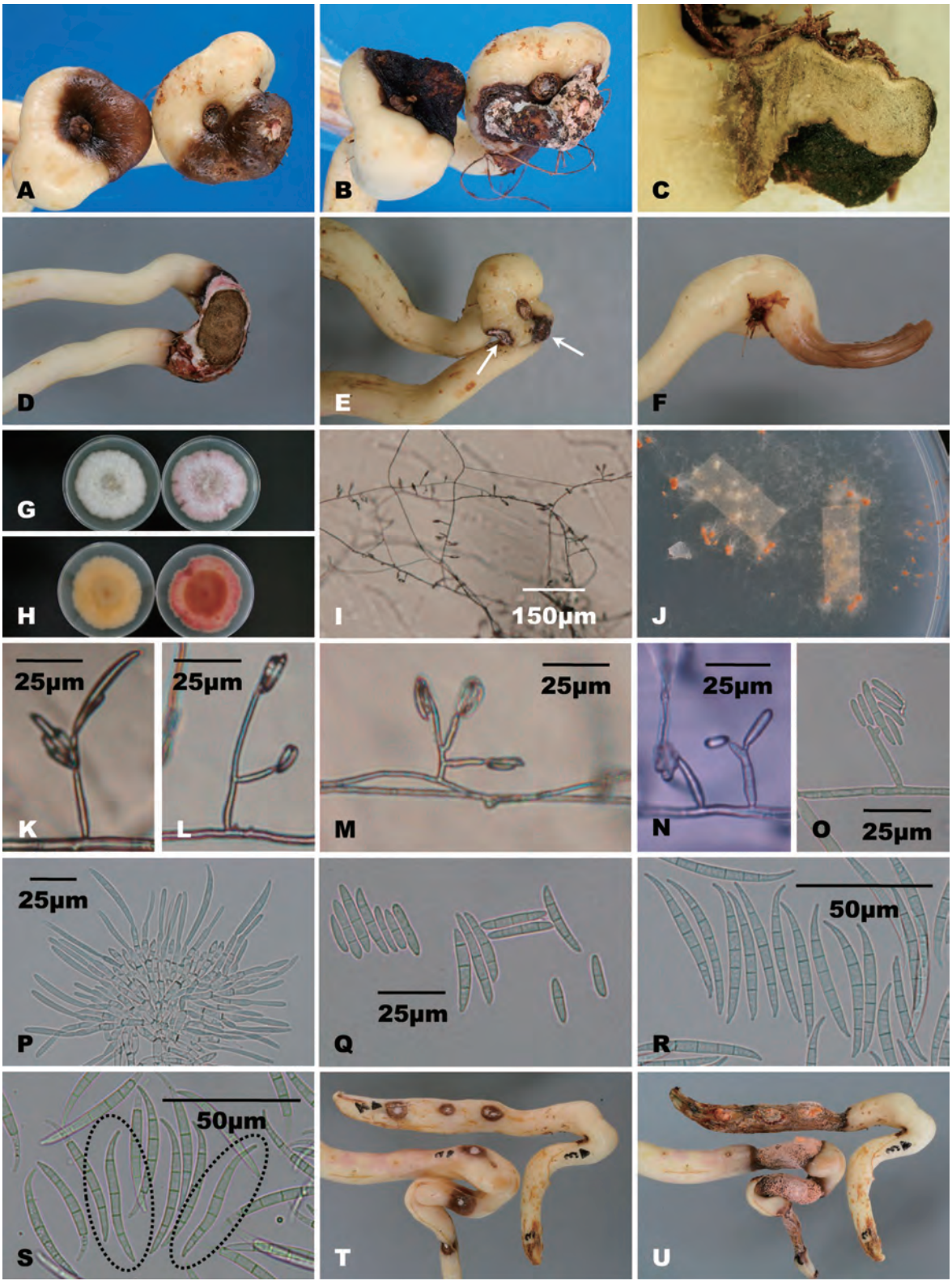

Fig. 1. A–F: Natural symptoms on *S. aurantiaca* caused by *F. anguioides* (A–E) and *Fusarium* sp. (A, F) (A: mixed with infection). B: Shrunken and discolored to black lesions six days after inspection of Fig. 1 A. C: Section of Fig. 1 A. G, H: Colony of the isolates (right: *Fusarium* sp. No. IM367-1-R, left: *F. anguioides* No. IM369-1-Y) on PDA at 25°C in the dark for 12 days (G: surface, H: reverse). I-S: Morphology of *F. anguioides*; aerial conidiophore with monophialide (K–M) and polyphialide (N, O) and conidia (K–N: No. IM369-1-Y, O, Q: IM100-2-Y). J: Orange sporodochia on agar surface. P–S: Sporodochial conidiophore (P), falcate conidia (R), and anguiform ones (S). (J, S: No. IM369-1-Y. P, R: No. IM210-A-Y). T, U: Symptoms on bulbs 3 days (T) and 13 days (U) after inoculation with isolates (upper: No. IM210-A-Y, lower: No. IM210-B-Y).

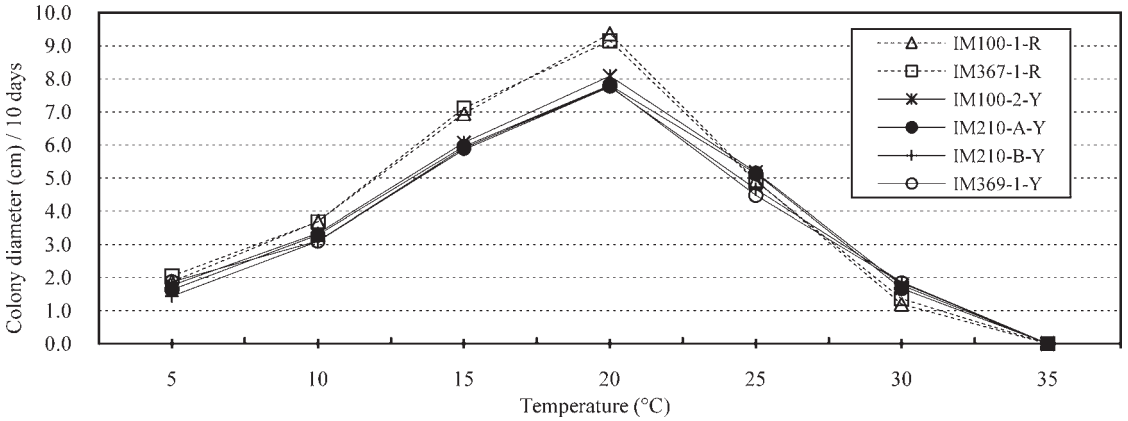

Fig. 2. Mean mycelial growth of six isolates at seven different temperatures (℃) on PDA in the darkness for 10 days.

Morphological characteristics

The isolates were incubated on SNA with several pieces of sterile filter paper (ca. 0.5×2 cm) at 20°C both in the dark and under continuous black light. In the yellow-type isolates under darkness, sporulation was initially observed within a few days in the aerial mycelium (Fig. 1 I) as single conidium or in false heads, and later, orange sporodochia were formed on the agar surface in about 25 days (Fig. 1 J). The primary (= aerial) conidiophores (Fig. 1 K–O) arose as lateral branches of hyphae, unbranched or sometimes branched, not proliferating sympodially, with holoblastic or phialidic conidiogenous cells, rarely with 2 conidiogenous loci (Fig. 1 N, O). The secondary (=sporodochial) conidiophores (Fig. 1 P) formed from substrate, branching loosely with ampuliform monophialide, 7.5–20×2.5–5.0 µm. Aerial conidia were mostly elliptical to clavate, fusoid 0–3(–5) septate, 11.3–39.5×2.5–5.5 µm (1–3 septate conidia), and sometimes falcate, 3(–5) septate without a pedicellate (foot-shaped) basal cell, 23.0–45.0×3.0–5.0 µm and 3–5 septate, with a pedicellate basal cell, 22.5–48.0×3.0–5.0 µm (Fig. 1 Q). Sporodochial conidia were falcate, slightly bent to anguiform, slender, tapering toward both ends, with an elongated, elegantly curved apical cell and pedicellate basal cell, 3–5 septate, 36.3–63×2.5–5 µm (5 septate conidia) (Fig. 1 R, S). No chlamydospores were observed. In contrast, elliptical to clavate, fusoid conidia were rare, and only falcate conidia with 3–5(–7) septa were abundantly produced under continuous black light. Morphological characteristics of the red-type isolates closely resembled the yellow-type isolates under both light conditions, except for sporodochial conidia not formed in the dark. Dimensions of conidia produced by the six isolates are compared in Table 1.

Pathogenicity test

The original host (*S. aurantiaca* bulbs and potted plants) and the bulbs of two kinds of other Liliaceous plants, tulip (*Tulipa* sp.) and glory-of-the-snow (*Chionodoxa* sp.), along with three kinds of Iridaceous plants, crocus (*Crocus* sp.), iris (*Iris* sp.), and freesia (*Freesia* sp.), were used for the experiment. The bulbs were used after surface sterilization with 70% ethanol. The potted plants were grown in a greenhouse after cold treatment of the bulbs. Inoculation was made by placing agar blocks (size 5×5×3 mm) containing mycelia from SNA cultures of the isolates onto the surface of a needle-wounded bulb, leaf, and stem. An aseptic SNA agar block was placed as a negative control. The inoculated bulbs and the potted plants were held in a plastic case with a cover to maintain humidity for 2–3 days, then uncovered and kept in the case at room temperature. Two to five days after inoculation, a similar symptom was reproduced and then progressed on the bulbs of the original host (Fig. 1 T, U). Severe leaf and stem blight were produced on the *S. aurantiaca* potted plants. Among the other bulbs tested, brown discoloration appeared only on the tulip. Inoculated fungi were reisolated from each lesion.

Phylogenetic analysis

DNA sequences of the partial β-tubulin gene and the translation elongation factor 1 alpha (TEF-1α) gene were determined to analyze phylogenetic and taxonomic positions of the isolates. These regions were amplified with the primer T1/T22 (O'DONNELL and CIGELNIK, 1997) and EF1/EF2 (O'DONNELL *et al.*, 1998), directly sequenced, and then compared with those of species belonging to the *Fusarium avenaceum* species complex and other groups reported by

Table 1. Comparison of dimensions of conidia produced by two kinds of six isolates causing bulb rot of *Sandersonia aurantiaca* with those of three *Fusarium* species.

Light conditions[*1]	Darkness					Continuous black light
Conidial type	Aerial conidia			Sporodochial conidia		
shape	elliptical to clavate, fusoid			falcate (to anguiform[*2])		falcate
septa	aseptate	1-septate	3-septate	3-septate	5-septate	5-septate
Red-type isolates						
IM100-1-R	7.5–16.3 × 2.5–3.8[*3] (12.8 × 2.8)	13.8–27.5 × 3.0–4.5 (20.0 × 3.4)	21.3–38.8 × 3.8–5.0 (29.8 × 4.6)	–[*4]	–	50.0–78.8 × 3.8–5.5 (64.3 × 4.2)
IM367-1-R	8.0–17.5 × 2.5–3.8 (12.9 × 2.8)	13.8–25.0 × 3.0–5.0 (19.7 × 3.5)	23.0–40.0 × 3.0–5.0 (30.2 × 4.3)	–	–	50.0–77.5 × 3.8–5.0 (64.0 × 4.1)
Yellow-type isolates						
IM100-2-Y	10.0–19.5 × 2.5–4.5 (12.3 × 3.8)	13.0–23.0 × 2.5–5.0 (17.8 × 3.8)	20.0–39.5 × 3.8–5.5 (28.4 × 4.7)	28.0–48.8 × 3.0–5.0 (37.5 × 4.3)	36.3–63.0 × 3.0–5.0 (48.8 × 4.0)	55.0–76.3 × 3.0–5.0 (68.4 × 4.1)
IM210-A-Y	8.0–15.0 × 2.5–3.8 (11.9 × 2.9)	11.3–23.8 × 2.5–5.0 (17.7 × 3.7)	20.0–35.5 × 3.8–5.0 (26.5 × 4.6)	28.8–48.0 × 2.5–5.0 (40.3 × 4.1)	39.5–60.5 × 2.5–5.0 (49.9 × 3.9)	52.5–70.5 × 3.8–5.0 (63.5 × 4.8)
IM210-B-Y	6.3–15.5 × 2.5–4.5 (11.8 × 3.0)	12.5–24.5 × 2.5–4.5 (17.6 × 3.5)	20.0–33.0 × 3.8–5.0 (27.0 × 4.5)	28.8–49.5 × 3.0–5.0 (40.2 × 4.2)	38.0–62.5 × 3.0–5.0 (49.5 × 4.1)	45.5–77.5 × 3.8–5.0 (60.1 × 4.5)
IM369-1-Y	7.0–18.0 × 2.5–3.8 (11.0 × 2.9)	11.3–22.0 × 2.5–4.5 (16.6 × 3.4)	20.5–30.0 × 3.8–5.0 (24.3 × 4.1)	32.0–53.8 × 3.0–5.0 (40.7 × 4.0)	38.0–60.0 × 3.0–5.0 (50.4 × 4.1)	54.5–75.5 × 3.8–4.5 (64.3 × 4.0)
(Total range)						
Red-type isolates	7.5–17.5 × 2.5–3.8	13.8–27.5 × 3.0–5.0	21.3–40.0 × 3.0–5.0	–	–	50.0–78.8 × 3.8–5.5
Yellow-type isolates	6.3–19.5 × 2.5–4.5	11.3–24.5 × 2.5–5.0	20.0–39.5 × 3.8–5.5	28.0–53.8 × 3.0–5.0	36.3–63.0 × 2.5–5.0	45.5–77.5 × 3.0–5.0
Fusarium anguioides[*5]	ND[*6]	20–38 × 3.9–5.3[*7] (27 × 4.4)		ND	33–80 × 3.0–5.4 (55 × 4.2)	ND
Fusarium avenaceum var. *avenaceum*[*5]	ND	18–22 × 2.8–3.2 (20 × 3.1)	25–30 × 4.0–4.2 (28 × 4.3)	45–50 × 3.2–3.8 (46 × 3.6)	48–65 × 3.0–4.0 (58 × 3.4)	ND
Fusarium arthrosporioides[*5]	ND	15–20 × 2.5–3.8 (17 × 3.2)	26-30 × 3.8–4.0 (27 × 4.0)	40-46 × 3.0–3.5 (45 × 3.3)	54–60 × 3.3–3.8 (56 × 3.5)	ND

[*1] The isolates were incubated on SNA at 20°C in the dark and under continuous black light.
[*2] Anguiform conidia were produced only by yellow-type isolates and *F. anguioides*.
[*3] Conidial dimensions: range and mean values of length × width (μm).
[*4] Not produced.
[*5] GERLACH and NIRENBERG (1982).
[*6] Not described.
[*7] Total range of 1–3-septatate conidia.

YLI-MATTILA *et al*. (2002) and KRISTENSEN *et al*. (2005), respectively. However, the sequence data on fungi whose identity is in doubt based on their morphological reexamination (YLI-MATTILA *et al*., 2002) were excluded from the current study. The partial β-tubulin sequences of all four isolates tested had 100% (408/408 bp) similarity with three species [*F. anguioides* Sherb. BBA 63598, *F. arthrosporioides* Sherb. BBA 71186, *Gibberella avenacea* R. J. Cook BBA 64151 (DNA Data Bank of Japan accession No. AF405448)]. The neighbor-joining (N-J) tree based on the sequence of this region showed that all four isolates tested fell into the monophyletic clade supported by 99% bootstrap value with these three species and were clearly separated from *F. tricinctum* (Corda) Sacc., *F. reticulatum* Montagne, and *G. acuminata* Wollenw. within the *Fusarium avenaceum* species complex, and also from *F. oxysporum* Schlecht. and *F. redolens* Wollenw. (Fig. 3). The N-J tree based on the partial TEF-1α sequences also showed that all four tested isolates formed a group together with the species within the *Fusarium avenaceum* species complex, e.g. *F. avenaceum* and *F. arthrosporioides* (no sequence data of *F. anguioides* exists in Genbank). However, the yellow-type isolates had 98% (635/646 bp) similarity with the red-type isolates, and they fell into a different clade (data not shown).

Identification

Based on septate, falcate conidia with a pedicellate basal cell and phialidic conidiogenesis, all isolates tested were considered to belong to the genus *Fusarium*, especially related to *F. avenaceum* and its allies, which have fusoid, apedicellate, blastic conidia over 30 μm long (PASCOE, 1990). The result was supported by phylogenetic analysis of the sequence of the partial β-tubulin and TEF-1α gene, and the current isolates were compared with the given descriptions of the three related species, *F. anguioides*, *F. arthrosporioides*, and *G. avenacea* [anamorph: *F. avenaceum* (Fr.) Sacc.] (Tables 1, 2). The morphological and cultural characteristics of the yellow-type isolates agreed with those of *F. anguioides* better than those of *F. avenaceum* var. avenaceum and *F. arthrosporioides*, especially with the width of sporodochial conidia (more than 4 μm), presence of anguiform conidia and orange sporodochia in color, colony diameter (ca. 5.0 cm/10 days), and absence of pyriform

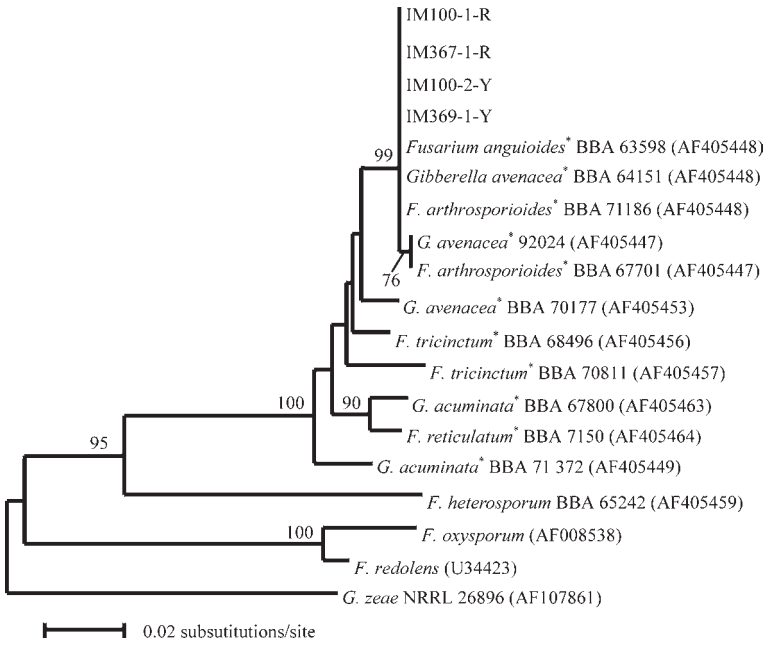

Fig. 3. A neighbor-joining phylogenetic tree of the species belonging to the *Fusarium avenaceum* species complex, including four isolates from *Sandersonia aurantiaca* bulbs based on the sequence of the partial β-tubulin gene. Numbers on the branches are bootstrap values in 1000 bootstrap replicates. The bootstrap values greater than 70 are shown. The accession numbers in the DNA Data Bank of Japan (DDBJ) are shown in parentheses. *The species belonging to the *Fusarium avenaceum* species complex.

Table 2. Comparison of main characteristics of two kinds of isolates causing bulb rot of *Sandersonia aurantiaca* with those of three *Fusarium* species.

	Yellow-type isolate (IM369-1-Y)	Red-type isolate (IM100-1-R)	*F. anguioides*[*1]	*F. avenaceum* var. *avenaceum*[*1]	*F. arthrosporioides*[*1]
Colony (PDA, 25°C, dark) color in reverse	yellowish to ocher	rose to carmine	cream, pink, rose to carmine, or yellowish to ocher	cream, pink, rose to carmine with yellowish to ocher tinges	cream, pink, rose to carmine, or purple with yellowish to ocher tinges
diameter (cm/10 days)	3.8–4.8 (avg. 4.5)	4.2–5.4 (avg. 4.9)	4.8–5.3	5.0–8.0	9.4–10[*2]
Conidia (SNA, 20°C, dark)					
anguiform (5-septate)	+	−	+	−	−
pyriform (0-septate)	−	−	−	−	+
Sporodochial color (SNA, 20°C, dark)	orange	−	orange to cinnamon or brick	bright orange	pale brick

[*1] GERLACH and NIRENBERG (1982).
[*2] 7.5–8.0 cm/8 days (original description) were recalculated for comparison.

conidia (Table 2). In conclusion, they were identified as *Fusarium anguioides* Sherb. On the other hand, the red-type isolates mostly corresponded morphologically with the yellow-type isolates (Tables 1, 2), and represented identical pathogenicity to *S. aurantiaca* and DNA sequences of the partial β-tubulin gene. However, they formed no sporodochial conidia, including anguiform conidia, under the dark condition, and represented different cultural characteristics and sequences of the partial TEF-1α gene than the yellow-type isolates. No possible species name could be found for the red-type isolates. Therefore, the red-type isolates were not identified and kept as *Fusarium* sp. within the *Fusarium avenaceum* species complex.

Fusarium anguioides Sherb. was first described as one of the dry rot pathogens of potato tubers in the United States (SHERBAKOFF, 1915) and of peas (*Pisum sativum* L.) in Japan (TOGASHI, 1928). But later, this species was considered a variety or synonym of *F. avenaceum* (CABI, 1951–1960; BOOTH, 1971). On the other hand, neotypification of the species was reported (NELSON *et al.*, 1995). Now this fungus is included in the *Fusarium avenaceum* species complex, which is composed of morphologically similar species, and the taxonomic status of each species, including *F. anguioides* and *F. avenaceum* s. str., is under discussion (YLI-MATTILA *et al.*, 2002). In our study, *F. anguioides* was treated as a different species from *F. avenaceum* especially based on the morphology of sporodochial conidia (GERLACH and NIRENBERG, 1982).

Name of the disease

Bulb rot of *S. aurantiaca* caused by the genus *Fusarium* is known as serious disease needed to be controlled in

Japan (SUMII and KOIKE, 1994). But the causal fungi have not yet been identified and are considered to be similar to *F. moniliforme* or *F. proliferatum* and *F. oxysporum* (HOSHI, 2004). In New Zealand, *Nectria haematococca* Berk. & Broome (anamorph: *F. solani*) (ANONYMOUS, 2002) and *F. oxysporum* Schltdl. (LANDCARE RESEARCH, 2010) were reported on this plant. The taxonomic positions of these *Fusarium* are clearly different from that of each species within the *Fusarium avenaceum* species complex. This is the first report of disease caused by *F. anguioides* and *Fusarium* sp. (within the *Fusarium avenaceum* species complex) on *S. aurantiaca* in Japan. We propose to name this new disease bulb rot (Japanese name: *kampu-byo*).

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和 文 摘 要

輸入検疫で発見されたサンダーソニア乾腐病（新称）

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2005～7年6～8月、成田国際空港における輸入検疫で、ニュージーランド産サンダーソニア球根に基部が乾腐し、褐～黒変したものを認めた。被害部位より*Fusarium*属菌が高率に分離され、有傷接種でサンダーソニア球根に原病徴を再現したほか、同・茎葉やチューリップ球根にも病原性を示し、各々から接種菌が再分離された。分離菌は、暗黒・25°CでPDA培地を黄変し、菌叢直径は4.5–5.2 cm/10 days、菌糸生育適温は20°Cである。また、本菌は暗黒・20°CのSNA培地上で、気生分生子は紡錘～棍棒型で隔壁数0–3(–5)、大きさ11.3–40 × 2.5–5.5 µm（1–3隔壁）及び鎌型、隔壁数3–5で脚胞のあるものとないものの両方があり、分生子座性分生子は先端が嘴状で中央は*F. avenaceum*に比べやや幅広く、緩く湾曲した鎌形で時にanguiform、隔壁数3–5で明瞭な脚胞を持ち、大きさ36.3–63 × 2.5–5 µm（5隔壁）、いずれも主にフィアロ型に生じる。以上から*F. anguioides*（Gerlach & Nirenberg, 1982）と同定した。また、PDA培地を赤変し、暗黒下で分生子座性分生子を形成しない菌株が存在し、これを*Fusarium* sp.とした。いずれもβ-tubulin およびTEF-1α 領域（一部）の解析から広義の*Fusarium avenaceum* species complex（種複合体）に所属することが示された。*F. anguioides*および*Fusarium* sp. によるサンダーソニアの病害は我が国未報告のため、本病の病名を乾腐病（Bulb rot）と提案する。