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Short Communication

Gray mold of Kalanchoe and Arabis caused by *Botrytis cinerea* intercepted in plant quarantine

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Abstract: Gray mold of Kalanchoe and Arabis were found at plant quarantine inspection in Yokohama, in 1997. Diseased plants showed dark, water-soaked lesions on leaves and stems and were covered with the characteristic gray and fuzzy sporulation. Isolated fungi were pathogenic to the original host plant and identified as *Botrytis cinerea* PERSOON:FRIES on the basis of their morphological characteristics. This is the first report describing a arabis disease caused by *B. cinerea* in Japan. Therefore, gray mold of arabis was proposed for the disease.

Key words: *Botrytis cinerea*, Kalanchoe, Arabis, Identification

In May of 1997, serious diseases of leaf and stem rot of potted kalanchoe(*Kalanchoe* sp.) and arabis (*Arabis blepharophylla* HOOK. *et* ARN.) were found at plant quarantine inspection in Yokohama. Those plants were transported in a reefer freight container from Denmark by sea. A *Botrytis* species was isolated at a high rate from them, and reproduced the same symptoms after inoculation with the fungi. From the size of conidia and the shape of conidiophores, the fungi were identified as *Botrytis cinerea* PERSOON:FRIES. This is the first to describe a gray mold disease of arabis in Japan.

Symptoms.

The main diagnostic symptoms of kalanchoe were characterized by dark, water-soaked lesions on leaves, stems and flowers. Infected plant were covered with white mycelia with characteristic gray, fuzzy sporulation under humid conditions (Fig.1). Lower leaves of infected

Fig. 1. Diseased kalanchoe caused by *Botrytis cinerea*.

Fig. 2. Diseased arabis caused by *Botrytis cinerea*.

arabis became yellowish brown and then decayed whole plant (Fig.2). Diseased leaves were covered with white mycelia with sporulation.

Isolation of the fungus.

A suspension of conidia in distilled water was prepared for the single spore isolation using 1.5% water agar (WA) medium. The conidia germinated readily on WA at 25℃. The isolates were cultured and stored on potato sucrose agar (PSA: 200g potato, 20g sucrose, 15g agar per liter). Two representative isolates (DK-2 from kalanchoe and DA-2 from arabis) were then chosen for subsequent morphological descriptions and inoculation tests.

Morphology of the fungi.

DK-2: The conidiophore of the fungus was upright with septa and light brown branches in the upper potion (Fig.3-A). The conidia were hyaline, ellipsoid to ovoid, 10.0-18.7×5.0-10.0 μm in size (L/B ratio:1.83).

DA-2: The conidiophore of the fungus was straight, brown and alternately branched (Fig.3-B). The conidia were hyaline, ellipsoid to ovoid, 8.7-12.5 × 6.2-10.0 μm in size (L/B ratio:1.47).

Scanning electron microscope (SEM) was applied to observe shape and surface structures of conidia of both isolates (DK-2 and DA-2). Conidial surface of both isolates was rough and warty (Fig.4). These surface structures were similar to those of *B. cinerea* reported by

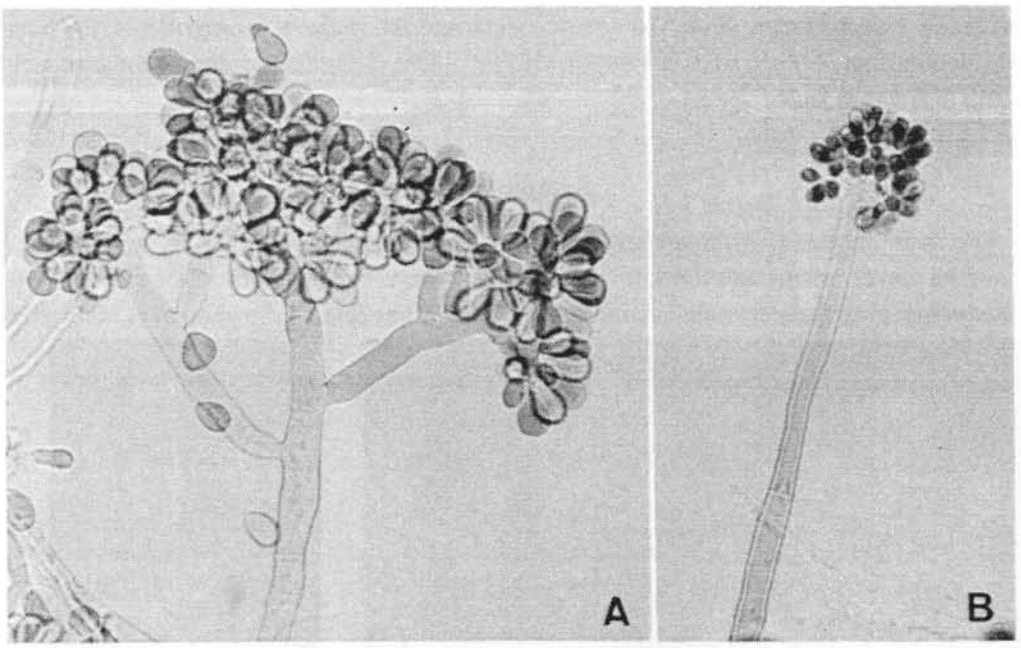

Fig. 3. Conidiophores and conidia of *Botrytis cinerea* from kalanchoe (A) and from arabis (B)(× 670).

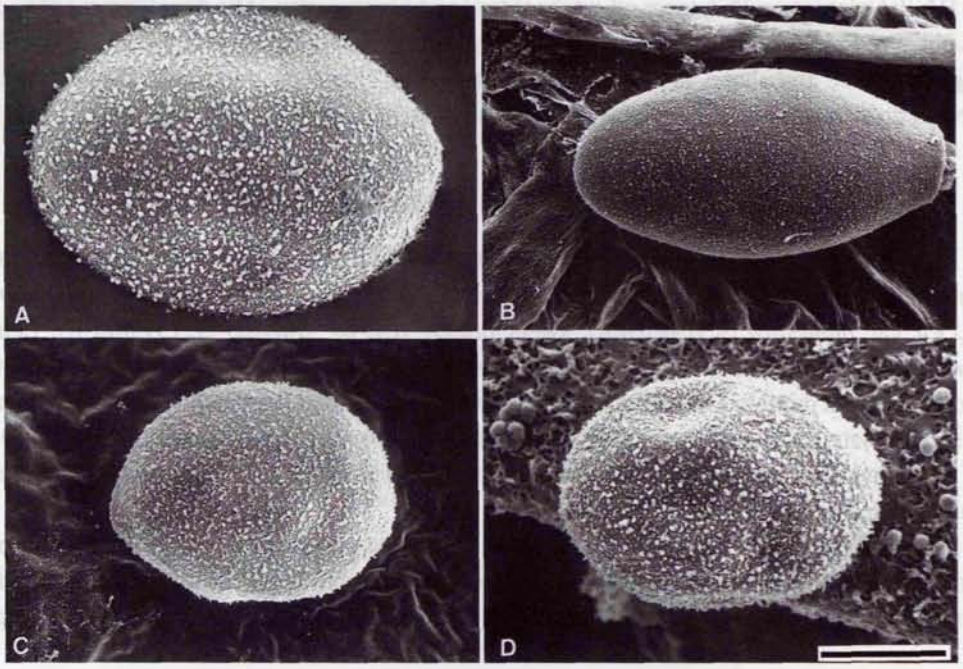

Fig. 4. SEM of surface structure of conidia of *Botrytis cinerea* from kalanchoe (A, B) and from arabis (C, D) (scale: 3μm).

KUSAKARI *et al.* (1997) and HORIUCHI *et al.* (1978).

The teleomorph stage of both fungus was not observed on sclerotia.

Culture note.

DK-2: Colonies on PSA were gray to brown with the development of spores (Fig.5-A). Sclerotia were black, irregular in size and shape, approximately 1-2 mm long, developed at the margin of a petri dish. Optimum temperature for mycelial growth was 20-25 ℃. No growth was seen at 35 ℃.

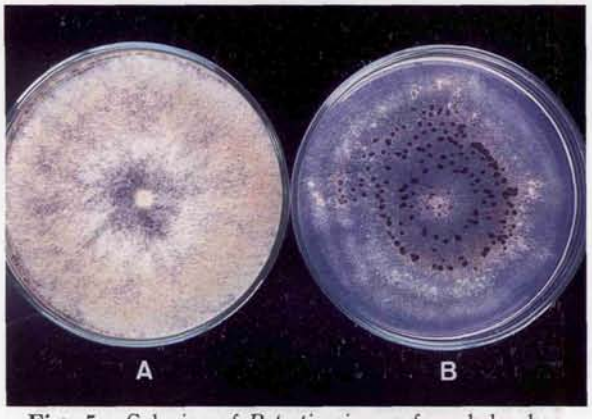

Fig. 5. Colonies of *Botrytis cinerea* from kalanchoe (A) and from arabis (B) on PSA at 25℃ for 2 weeks.

DA-2: Colonies on PSA were off-white at first and became gray. The development of spores was observed scatteredly on PSA. Sclerotia were black, tightly appressed to the agar medium, irregular in size and shape, approximately 3-4 mm long (Fig.5-B). Optimum temperature for mycelial growth was 15-20 ℃. No growth was seen at 35 ℃.

Pathogenicity of the fungi.

Pathogenicity was tested at least twice to the following plant: kalanchoe (*Kalanchoe* sp.), arabis (*A. blepharophylla*), strawberry (*Fragaria* × *ananassa* DUCH.), apple (*Malus pumila* MILLER var. *domestica* SCHNEIDER), sweet orange (*Citrus sinensis* (L.) OSBECK), Lemon (*Citrus limon*(L.) BURM.f.). The plant surface was wounded with a cork borer or a bundle of 20 needles (2-3 mm depth). Mycelial mats on PSA were placed onto the wounded and/or unwound parts. Inoculated plants were maintained in moist condition at room temperature (20-25℃) for 2-5 days and then placed in a glasshouse for 2-3 weeks or kept indoors for 1 week. Noninoculated plants served as controls.

Results are shown in Table 1. DK-2 caused water-soaked lesions on kalanchoe leaves, fruit rot of strawberry and brown rot lesions on sweet orange and lemon fruit. Three days after inoculation, strawberry fruit were covered with mycelia with gray and fuzzy sporulation (Fig.6-

Table 1. Pathogenicity of *B. cinerea* from Kalanchoe (DK-2) and from Arabis (DA-2).

Plant	Part inoculated		DA-2	DK-2
Kalanchoe	leaf	wound	+ a)	+
		unwound	+	+
Arabis	leaf	wound	+	−
		unwound	−	−
Strawberry	fruit	wound	+	+
		unwound	+	+
Apple	fruit	wound	+	+
Orange	fruit	wound	+	+
Lemon	fruit	wound	+	+

a) +, positive; −, negative for disease development.

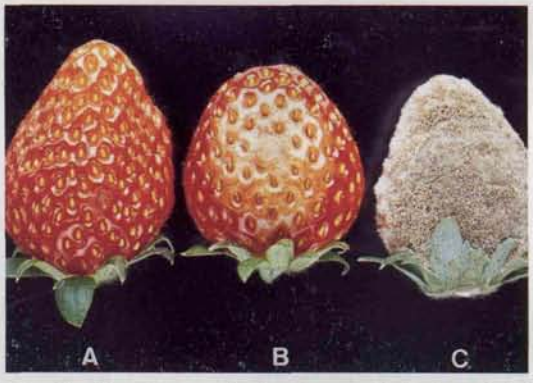

Fig. 6. Symptoms of strawberry fruit inoculated with *Botrytis cinerea* DA-2 (B) and DK-2 (C). Left fruit (A) is a noninoculated one saved as control.

C). No symptoms was observed on arabis leaves inoculated with DK-2.

DA-2 caused light brown lesions on wounded arabis leaves, water-soaked lesions on kalanchoe leaves and brown rot on sweet orange and lemon fruit. Inoculated strawberry fruit showed light brown lesions with scattered sporulation(Fig.6-B). Control leaves and fruit showed no symptoms.

Identification of the causal fungi.

The morphological characteristics of the fungal conidiophores and conidia of both isolates close resembled to those of *Botrytis cinerea* (ELLIS, 1971). Size of conidia formed on three substrates (original host plant, PSA and strawberry fruit) was compared (Table 2). Both isolates (DK-2 and DA-2) were identified as *Botrytis cinerea* PERSOON:FRIES based on the size of conidia and the shape of conidiophores (Table 3). *B. cinerea* was reported as causal fungus of gray mold of kalanchoe in Japan (TAKEUCHI *et al.*, 1995).

There are some difference between DK-2 and DA-2 in pathogenicity to arabis leaves, in optimum temperature for myceril growth and in symptoms on strawberry fruit. Gray mold of arabis caused by *B. cinerea* has been known in the USA (FARR *et al.*, 1995). This is the first report of a arabis disease caused by *B. cinerea* in Japan. Therefore, the name "Gray mold" is proposed for the disease.

Table 2. Comparison of conidial size of *B. cinerea* (DK-2 and DA-2) on host plant, PSA and strawberry fruit.

Substrate	DA-2	DK-2
Original host plant	8.7-12.5 × 6.2-10 μm	10-18.7 × 5-10 μm
	(av. 10.7 × 7.6)	(av. 13.0 × 7.1)
	L/B:1.7	L/B:1.83
PSA	7.5-12.5 × 5-7.5	10-18.7 × 7.5-10
	(av. 9.6 × 6.7)	(av. 14.8 × 8.9)
	L/B:1.43	L/B:1.66
Strawberry fruit	8.7-12.5 × 5-8.7	10-17.5 × 6.2-10
	(av. 10.9 × 7.4)	(av. 12.2 × 8.0)
	L/B:1.47	L/B:1.52

Table 3. Comparison of conidial size among DK-2, DA-2 and *B. cinerea* reported previously.

Isolate	Size	L/B ratio
DA-2	8.7-12.5 × 6.2-10	1.47
DK-2	10-18.7 × 5-10	1.83
B. cinerea [a)]	6-18 × 4-11	1.35-1.5
B. cinerea [b)]	8-16 × 5-10	

a) ELLIS (1971)

b) KUSAKARI *et al.* (1997)

Literature cited

ELLIS, M. B. (1971) *In* Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, PP. 178-184.

FARR, D. F., BILLS, G. F. CHAMURIS, G. P. and ROSSMAN, A. Y. (1995) *In* Fungi on Plants and Plant Products in The United States. pp.108-109.

HORIUCHI, S. HORI, M. and ISHII, M. (1978) Identification of Genus *Botrytis* with Morphological Structures of Fungal Organs by Scanning Electron Microscope. Bulletin of The Chugoku National Agricultural Experiment Station E**13**:53-87.

KUSAKARI, S., OKADA, K. and KAWARADANI, M. (1997) Gray mold of clematis caused by *Botrytis cinerea*. Ann. Phytopathol. Soc. Jpn. **63**:399-402.

TAKEUCHI, J., HORIE, H. and HIRANO, T. (1995) Gray mold of some garden plants in Tokyo is caused by *Botrytis cinerea*. Proceedings of the Kanto Tosan Plant Protection Society **42**:105-107.

和 文 摘 要

輸入検疫で発見されたカランコエ灰色かび病およびアラビス灰色かび病（新称）

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1997年5月，横浜港での輸入検疫においてデンマーク産カランコエ（*Kalanchoe* sp.）およびアラビス（*Arabis blepharophylla*）の苗に，株元に灰色のかびを生じ，茎葉が黄化萎凋する症状が認められた。被害植物はその後枯死し，罹病株上にはBotrytis属菌が観察された。常法により罹病植物から単胞子分離を行い，得られた菌株を原寄主に接種したところ，病徴が再現され発病株から接種菌が再分離された。分離菌の生育適温はカランコエ菌は20～25℃，アラビス菌は15～20℃であった。両菌の分生胞子柄は直立し，樹状に分岐する。分生胞子は無色，単胞，楕円形で，大きさはカランコエ菌10～18.8×5～10(av. 13×7.1) μm L/B比1.83，アラビス菌8.7～12.5×6.2～10(av. 10.7×7.6) μm L/B比1.4であった。両菌の形態をELLIS (1971) の記載と比較したところ，両菌とも*Botrytis cinerea* PERSOON:FRIESと同定された。本菌によるアラビスの病害は本邦未報告と思われるので，本病をアラビス灰色かび病（Gray mold of arabis）と呼称したい。