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Alternaria petasitis sp. nov., causing *Alternaria* leaf spot of Japanese butterbur in Japan

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We isolated an *Alternaria* species from brown leaf spots of Japanese butterbur in Kanuma city, Tochigi Prefecture in August 1996, and in Akagi village, Gunma Prefecture in June 1997. The isolated fungus formed irregularly shaped colonies with yellow-brown pigment on potato sucrose agar (PSA) and potato dextrose agar (PDA). When Japanese butterbur leaf was inoculated with small fragments of mycelia, brown spot lesions were produced. Conidial suspensions of the fungus also produced similar spots on Japanese butterbur leaf. Under moist conditions, a small amount of conidia was produced on the spots. The conidia were somewhat variable in shape and size, measuring 102–362 × 9–22 μm for the Kanuma isolate and 70–392 × 11–27 μm for the Akagi isolate. Length of the conidial beak was also variable, being 30–280 μm for the Kanuma isolate and 30–302 μm for the Akagi isolate. The fungus could not be identified with any of *Alternaria* species hitherto known, suggesting that it was new to science. We proposed to designate the fungus as *Alternaria petasitis*, giving a Latin description of the new species.

Key Words——*Alternaria petasitis* sp. nov.; Japanese butterbur; leaf spot.

Japanese butterbur [*Petasites japonicus* (Siebold & Zucc.) Maxim.] is a common native plant and its petiole is a traditional vegetable food in Japan. It has been grown in the field as an edible plant. We found a leaf spot disease of Japanese butterbur in Kanuma city in August 1996. Similar leaf spots were also found on the same plant in Akagi village, Gunma pref. in June 1997 (Fig.1). The spots were also seen on petioles of the plants. We isolated fungi responsible for these spots and the isolates seemed to be a species of *Alternaria*. In Japan, disease caused by *Alternaria* species has not been reported on Japanese butterbur (Kishi, 1998). The morphology and pathogenicity of the fungi are reported here.

Materials and Methods

Isolation of fungi and their characteristics on medium Pieces of leaf tissue were cut from the spotted area, placed on potato dextrose agar (PDA; Difco Co. Ltd.) or water agar (WA; 1.5%(w/v) agar) and incubated at 25°C. The fungi emerging from the infected tissues were isolated. HAK1, one of the isolates from Kanuma, and HAA1, from Akagi, were selected and grown on PDA or potato sucrose agar [PSA; 2%(w/v) sucrose and 1.5%(w/v) agar in broth of 20%(w/v) potato] plates for maintenance and experimental use. Mycelial disks (5 mm in diam) were incubated on PSA plates at various temperatures (10–40°C) in the dark and assessed for the colony diam. The fungi were also grown on PSA plates under natural light and black light blue (BL-B; 15W, National) at 25°C to induce conidial formation.

Pathogenicity and observation of conidial morphology Mycelial disks (5 × 5 mm) of the isolates were put on leaves of Japanese butterbur and globe amaranth (*Gomphrena globosa* L.) with or without wounding by cutting with a knife. The plants were then kept under moist conditions in a greenhouse for several days. The inoculated leaves were observed for disease development and conidial formation. Conidia formed and pieces of the inoculated leaves were incubated on WA at 24°C for fungal re-isolation. To test pathogenicity, the conidia produced on the inoculated leaves of Japanese butterbur were suspended in water, dropped onto leaves of Japanese butterbur and globe amaranth with or without wounding, and the plants were kept under moist conditions. Toxicity of the fungal secretion into PSA was assessed as follows. Agar disks (5 × 5 mm) from around the colonies of the isolates on PSA were removed and put on leaves of Japanese butterbur and globe amaranth with or without wounding, and the plants were kept under moist conditions. Filtrates of liquid culture media, 20%(w/v) potato broth with 2%(w/v) sucrose, in which the fungal isolates had been grown for 1 wk were dropped on leaves of Japanese butterbur and globe amaranth with or without wounding, and the plants were kept under moist conditions.

Results

Colony characteristics of the fungus on agar media The

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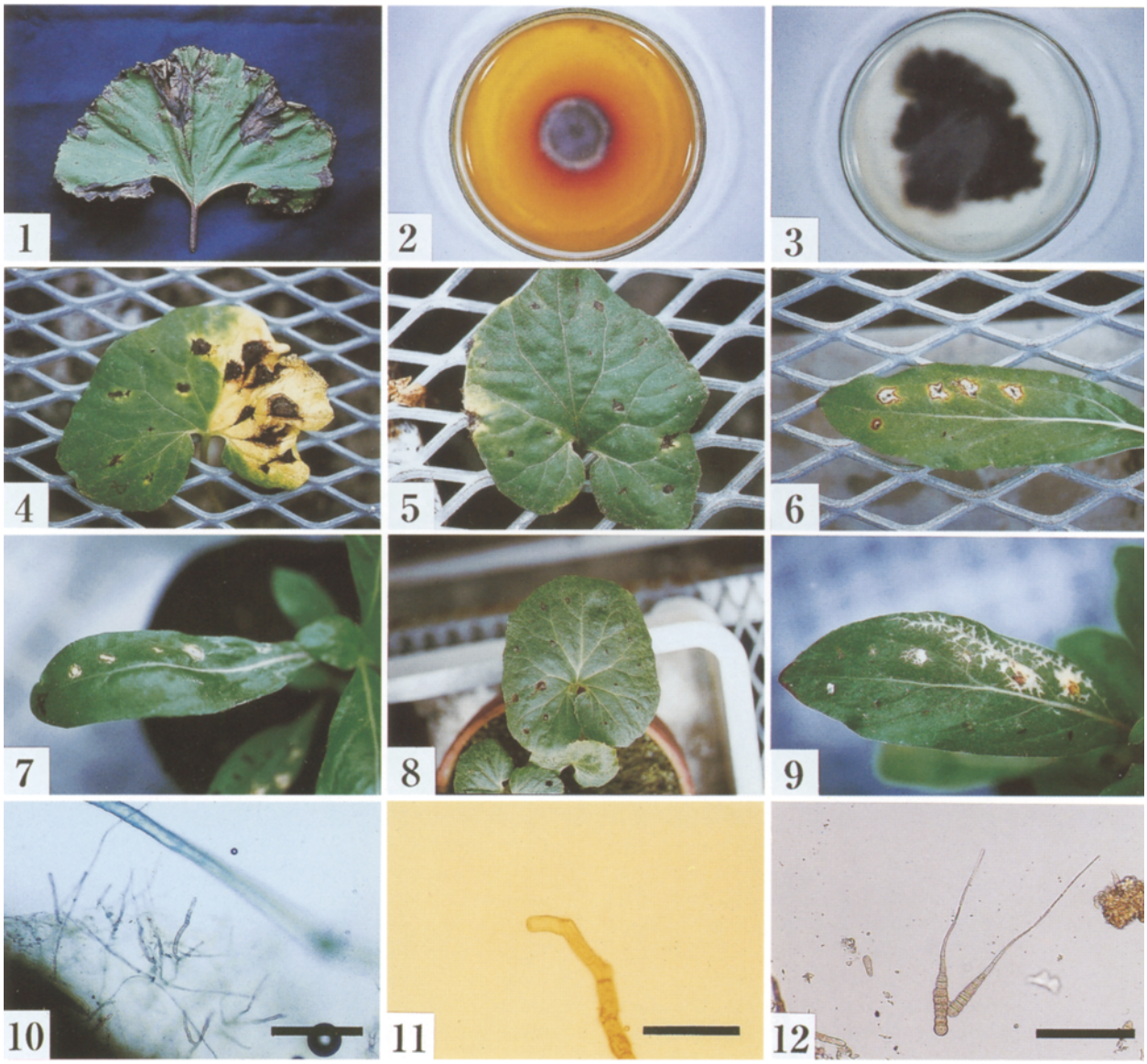

Figs. 1–12. Symptoms on leaves of Japanese butterbur and globe amaranth caused by *Alternaria petasitis* and its morphology. 1. Disease symptom on Japanese butterbur leaf caused by *A. petasitis* from field in Akagi. 2, 3. Colonies of *A. petasitis* on PSA after 1 wk of incubation at 24°C: isolate HAK1 (2) and isolate HAA1 (3). 4. Symptoms on Japanese butterbur leaf caused by *A. petasitis* HAK1, 14 d after mycelial inoculation, with (right) or without (left) wounding. 5. Symptoms on Japanese butterbur leaf caused by HAK1, 14 d after conidial inoculation, with (right) or without (left) wounding. 6. Symptoms on globe amaranth leaf caused by HAK1, 14 d after mycelial inoculation, with (upper) or without (lower) wounding. 7. Symptoms on globe amaranth leaf caused by HAK1, 14 d after conidial inoculation, with (upper) or without (lower) wounding. 8. Symptoms on Japanese butterbur leaf incited by agar disks cut from around HAK1 colony on wounded (left) or unwounded (right) portions. 9. Symptoms on globe amaranth leaf incited by agar disks cut from around HAK1 colony on wounded (upper) or unwounded (lower) portions. 10. HAK1 conidiophores on Japanese butterbur leaf, stained with cotton blue (scale bar: 200 μm). 11. Tip of HAK1 conidiophore (scale bar: 50 μm). 12. Conidia of HAK1 produced on Japanese butterbur leaf (scale bar: 100 μm).

fungus produced yellow-brown pigment on PSA and PDA, but not on WA (Figs. 2, 3). The degree of pigmentation of agar media varied between the isolates. The pigment was produced in the dark, as well as under natural light and BL-B. The colonies on PSA and PDA had wooly aerial mycelia and irregular margins. The colony color varied from gray to brown or black. Growth of the colonies on agar media was not stable, mutating in growth rate, degree of pigmentation of medium and colony color (white to livid). On PSA, the optimum temperature for growth was 25°C (Fig. 13). Conidia were not produced when the isolates were incubated in the dark, but a few conidia were produced under BL-B.

Pathogenicity When mycelia and conidial suspensions of both isolates were applied to leaves of Japanese butterbur with or without wounding, brown lesions ap-

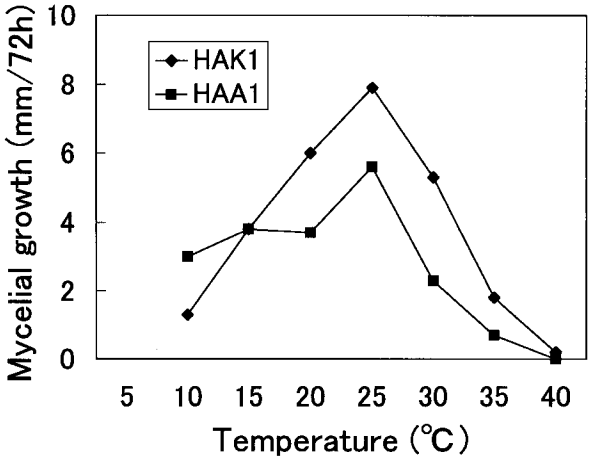

Fig. 13. Mycelial growth of *A. petasitis* isolates HAK1 and HAA1 on PSA at different temperatures.

peared and expanded in 3 d (Figs. 4, 5). Small amounts of conidia were produced under moist conditions 2 wk after inoculation. The same species of fungi were re-isolated from artificially produced lesions on Japanese butterbur leaves. In leaves of globe amaranth, whitish lesions appeared only on wounded portions, which expanded slightly (Figs. 6, 7) and produced a few conidia. Agar disks from the periphery of colonies on PSA or PDA plates gave rise to spots on wounded portions of Japanese butterbur and globe amaranth leaves (Figs. 8, 9). Filtrates of the fungal cultures, however, did not cause such spots on leaves of Japanese butterbur and globe amaranth.

Morphology Conidia were produced solely from the conidiophores that grew sparsely and branched one to three times near the base (Figs. 10, 14). The conidiophores were brown and geniculate with the scars (Fig. 11). Widths of the conidiophores of HAK1 and HAA1 were 5.5–10.5 (av. 7.5) μm and 4–8 (av. 7) μm, respectively. The conidia were narrow claviform to moniliform with a long beak, yellowish brown to dark brown, and had a few longitudinal septa in addition to cross septa (Figs. 12, 15). The conidia of HAK1 measured 102–362×9-22 (av. 220×17) μm with 2–10 (av. 6.5) main-body cells. Their beak was 30–280 (av. 153) μm long with 3–13 (av. 7.8) cells (Table 1). The conidia of HAA1 measured 70–392×11–27 (av. 225×19) μm with 2–13 (av. 7.1) main-body cells. Their beak was 30–302 (av. 152) μm long with 1–13 (av. 6.4) cells.

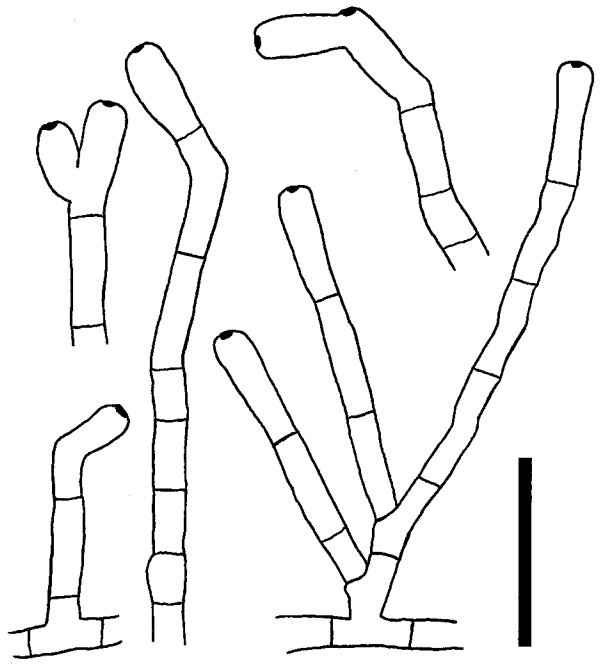

Fig. 14. Conidiophores of *A. petasitis* isolate HAK1 from Japanese butterbur leaf (scale bar: 50 μm).

Discussion

Pathogenicity of two fungal isolates from the leaf spots of Japanese butterbur from different locations was confirmed in inoculations. The spots on Japanese butterbur leaves caused by the isolates expanded, often resulting in blight of whole leaves. On globe amaranth leaves, spots were produced only on wounded leaves and did not expand widely from the site of inoculation. The toxic substance secreted into agar media by the fungus damaged both Japanese butterbur and globe

Table 1. Conidial morphology of *Alternaria* isolates from Japanese butterbur, *A. gomphrenae* and *A. longissima*.

Characteristics	HAK1[a]	HAA1[a]	*A. gomphrenae*[b]	*A. longissima*[c]
Length (μm)	102–362(220)[d]	70–392(225)	102–195(149.4)	54–470
Width (μm)	9–22(17)	11–27(19)	9–18(13.76)	4–17
Beak length (μm)	30–280(153)	30–302(152)	33–111(69.2)	N
Number of main-body cells	2–10(6.5)	2–13(7.1)	N[e]	N
Number of beak cells	3–13(7.8)	1–13(6.4)	N	N

a) Conidia produced on Japanese butterbur leaves.
b) Yoshii (1933).
c) Long conidia described by Deighton and MacGarvie (1968).
d) Averages in parenthesis.
e) Not described.

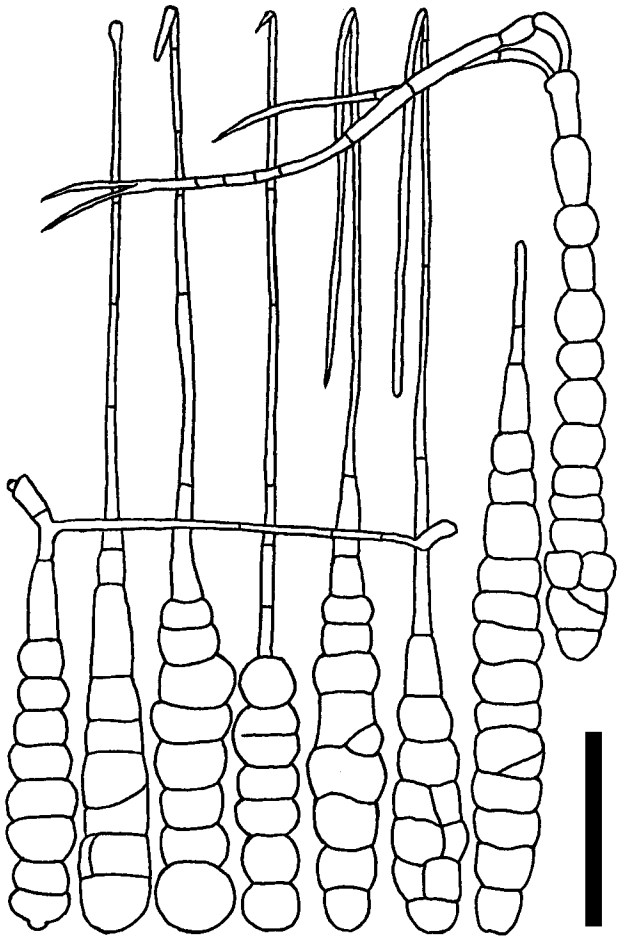

Fig. 15. Conidia of *A. petasitis* isolate HAK1 from Japanese butterbur leaf (scale bar: 50 μm).

amaranth leaves with wounding. Brown, claviform or moniliform poroconidia were produced solely from the brown conidiophores on the spot. The isolates were very similar to each other in conidial morphology (Table 1), and we identified the fungus as a species of *Alternaria* (Kobayashi, 1992). Colony characteristics of the isolates with yellow-brown pigmentation, gray aerial mycelia and scarce conidia resembled those of *A. gomphrenae* Togashi isolated from globe amaranth (Yoshii, 1933). *A. gomphrenae*, however, showed strong pathogenicity to globe amaranth and produced spots on leaves without wounding, and its conidia were smaller than those of our isolates (Togashi, 1926; Yoshii, 1933). *A. longissima* Deighton & MacGarvie, which was isolated from various grains, also has conidia with a long beak, although the pigmentation of media was not mentioned in the monographs (Deighton and MacGarvie, 1968; Ellis, 1971). *A. longissima* grew fast on PDA, and the conidia and conidiophores were narrower than those of our isolates (Deighton and MacGarvie, 1968; Ellis 1971). We could not find any other species similar to our isolates. Therefore, the present fungus was best considered as new, and we designed it as *Alternaria petasitis*.

Alternaria petasitis M. Kubota, Kishi et Abiko, sp. nov. Figs. 2, 3, 10–12, 14

Foliicola; maculis brunneis, irregularibus, 0.5–3 cm diam; conidiophoris brunneis, sparsis, prope basin 1– vel 3– plo ramificantibus, apice rotundatis et uniporosis, interdum geniculatis, 30–220 μm longis, 4–10.5 μm incrassatus; conidiis solitariis, pallide luteis vel atrobrunneis, laevibus, obclavatis, longirostratis, 10–30 μm latis, 70–400 μm longis, 2–13-cellularibus; rostris 30–300 μm longis, 1–13– cellularibus; septum longitudinale sparsum.

Holotypus: Fuki-AK1, on leaves and petioles of *Petasites japonicus* (Siebold & Zucc.) Maxim. ("Fuki" in Japanese), Kanuma, Tochigi Pref., August 1996, collected by M. Kubota. The specimen was deposited in the National Research Institute of Vegetables, Ornamental Plants and Tea.

Other specimen examined: Fuki-AA1, on leaves and petioles of *Petasites japonicus*, Akagi, Gunma Pref., June 1997, deposited in the National Research Institute of Vegetables, Ornamental Plants and Tea.

Living cultures of HAK1 from Kanuma and HAA1 from Akagi are preserved in the National Research Institute of Vegetables, Ornamental Plants and Tea.

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