

Aureobasidium microstictum (Bubak) W. B. Cookeによるカンゾウ類葉枯病 (新称)

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Leaf Blight of Day Lily Caused by *Aureobasidium microstictum* (Bubák) W. B. Cooke

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Abstract

Brown spots and yellow stripes were observed on leaves of *Hemerocallis fulva* var. *kwanso* at the herbal garden of Kyoto Prefectural Research Institute of Agriculture in Ayabe in the late June of 1985. In the beginning, small brown spots appeared on both sides of the leaves and then bright yellow stripes occurred and brownings expanded gradually from the leaf tips. These symptoms had been observed until mid-November. No pathogen was generally observed on diseased leaves, while acervuli composed of the tightly interwoven hyphae were formed on host plants in moist chamber. Conidiogenous cells were clavate to subcylindrical. Conidia were blastic, ellipsoidal to fusoid, hyaline, smooth, one-celled. Inoculation tests indicated that isolates were pathogenic on injured leaves of *H. fulva* var. *kwanso*, *H. fulva* var. *disticha*, and *H. fulva* var. *fulva*. An isolate from naturally infected *H. fulva* var. *disticha* showed the same pathogenicity as those from *H. fulva* var. *kwanso*. On PDA medium, single conidium isolates formed creamy and yeastlike colonies at first, then after formed white hyphae, and eventually turned dark brown to black in color. Growth on PSA medium occurred in the range from 8 to 30 C and optimum growth at between 20 and 24 C. Conidia formed on PDA medium were blastic, ellipsoidal to fusoid, hyaline, smooth, one-celled. Secondary conidia were formed and yeastlike growth appeared. On the basis of its morphological characteristics and pathogenicity on the plants of the genus *Hemerocallis*, the present fungus was identified as *Aureobasidium microstictum* (Bubák) W. B. Cooke, and a common name, leaf blight, was proposed.

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Key words : *Hemerocallis fulva* var. *kwanso*, *Aureobasidium microstictum*, leaf blight.

Introduction

Tawny daylily, *Hemerocallis fulva* L. var. *kwanso* Regel, that grows wild in fields and hills in Japan is a perennial plant of Liliaceae. It's spindle-shaped tuberous root called *Hemerocalli Radix* is utilized as a herb medicine and it's flower bud called *Hemerocalli Flos* is used for food¹⁾.

Disease of *H. fulva* var. *kwanso* other than rust has never been reported in Japan²⁾. In the late June of 1985, brown spots and yellow stripes on the leaves of *H. fulva* var. *kwanso* were found at the herbal garden of Chutan Branch, Kyoto Prefectural Research Institute of Agriculture. In this paper, we report that the present disease is a new disease caused by a species of the genus *Aureobasidium* hitherto unreported in Japan.

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Part of this work was presented at the Annual Meeting of the Phytopathological Society of Japan (Nagoya, 1986)⁸⁾.

Materials and Methods

Isolates. In July of 1985, leaves of *H. fulva* var. *kwanso* showing yellow stripe symptom and those of *H. fulva* L. var. *disticha* M. Hotta growing adjacently at the herbal garden were collected. After incubating in moist chamber for 24 hours, single conidium isolates were obtained on potato-dextrose agar (PDA) slants. The following three isolates were used throughout this study: CB-8533 (IFO 32065) and CB-8535 (IFO 32067) isolated from *H. fulva* var. *kwanso*, and CB-8534 (IFO 32066) from *H. fulva* var. *disticha*.

Inoculation. Inoculum of each isolates was prepared as follows: each isolates was incubated on PDA slants for 14 days at 25 C. After incubations, PDA slants were shaken vigorously adding each 3 ml of distilled water, and were stood for a few minutes. One ml of conidial suspensions thus obtained were permeated on 1 cm square distilled absorbent cotton. *Hemerocallis fulva* var. *kwanso*, *H. fulva* var. *disticha* and *H. fulva* L. var. *fulva* grown in clay pots (20 cm in diameter) were used for inoculation tests. Injured and non-injured inoculation were conducted by sticking the inoculum cottons with cellophane adhesive tapes each three on the test plants. Inoculated plants were kept at 25 C for 14 days in a growth cabinet.

Morphological observation. Generally, no pathogen was observed on diseased leaves, so leaves with symptom were incubated in moist chambers for 24 hours and made hand-sections of the leaves. The morphology of the pathogen was examined under a light microscope. Each isolates were slide-cultured on PDA at 25 C for 7 days for observation of morphology of the fungus on media. Colonies of each isolates were compared by culturing on potato-sucrose agar (PSA), malt agar (MA), oatmeal agar (OMA) and Czapek agar (CA) slants at 24 C for 14 days.

Optimum temperature for growth. Optimum temperature for growth were determined by incubating each isolates at 3, 8, 17, 20, 24, 28, 30 and 37 C for 14 days on PSA slants and MA slants.

Results

Symptoms

In mid-April, small brown spots appeared on both sides of the leaves of *H. fulva* var. *kwanso* (Plate I -1). In the early May, bright yellow stripes occurred toward the leaf tips (Plate I -2, 3). As the symptom progresses, brownings expanded from the leaf tips (Plate I -4). Eventually these leaves were killed. These symptoms were observed until the late July, but appeared not to develop in August and September, recurred in mid-October through mid-November. The same symptoms appeared on the leaves of *H. fulva* var. *disticha* in adjoining row.

Morphology of the pathogen on diseased leaves

Acervuli on both sides of the leaves were composed of tightly interwoven hyphae in host tissues (Plate II-5, III-11). Conidiogenous cells were clavate to subcylindrical, hyaline, smooth, densely packed, protruding through the acervuli, $30-40 \times 4-10 \mu\text{m}$ (means $36.4 \times 6.6 \mu\text{m}$). The blastic conidia were formed simultaneously at the apex and distinct scars remained there (Plate II-6, III-12). Conidia were ellipsoidal to fusoid, hyaline, smooth, one-celled, polyblastic, $5.5-18 \times 2-4.5 \mu\text{m}$ (means $9.6 \times 3.2 \mu\text{m}$). Secondary conidia were often formed (Plate II-7, III-13).

Pathogenicity

On injured leaves of *H. fulva* var. *kwanso* and *H. fulva* var. *fulva*, all three isolates produced small brown spots and bright yellow stripes. On injured leaves of *H. fulva* var. *disticha*, CB-8533 and CB-8534 produced only small brown spots, while CB-8535 produced small brown spots with yellow halo. No isolate produced symptom on non-injured leaves of test plants (Table 1).

Morphology of the pathogen on media

Acervuli were not formed on media. Hyphae were hyaline, smooth, $2-6.5 \mu\text{m}$ wide (mean $4 \mu\text{m}$). Brown, thin-walled hyphae were often observed. Conidiogenous cells were observed in terminal of hyphae. Polyblastic conidia were formed at the apex or on the side of the conidiogenous cells leaving distinct scars on them (Plate II-8, III-14). Conidia were ellipsoidal to fusoid, hyaline, smooth, one-celled, $5.5-20 \times 2-5 \mu\text{m}$ (means $9.4 \times 3.7 \mu\text{m}$). Secondary conidia were often formed blastically at one end or both ends of the conidia without forming conidiogenous cells and yeastlike growth appeared (Plate II-9, III-15). Chlamydospores were ellipsoidal, brown, $8-27 \times 4-15 \mu\text{m}$ (means $17 \times 7.9 \mu\text{m}$) (Plate II-10, III-16).

Characteristics of colonies on media

On PSA, MA and OMA, each isolates formed creamy, yeastlike colonies at first, then often formed white hyphae, and eventually turned dark brown to black in color; after incubation for 14 days, CB-8533 showed yeastlike growth, CB-8534 white hyphal

Table 1. Pathogenicity of *Aureobasidium microstictum* isolated from diseased leaves of *Hemerocallis fulva* var. *kwanso* and *H. fulva* var. *disticha*

Plant	Isolate					
	CB-8533 ^{a)}		CB-8535		CB-8534	
	Injured	Non-injured	Injured	Non-injured	Injured	Non-injured
<i>Hemerocallis fulva</i> L. var. <i>kwanso</i> Regel	++ ^{b)}	—	++	—	++	—
<i>Hemerocallis fulva</i> L. var. <i>disticha</i> M. Hotta	±	—	+	—	±	—
<i>Hemerocallis fulva</i> L. var. <i>fulva</i>	++	—	++	—	++	—

a) CB-8533 and CB-8535: Isolates from *H. fulva* var. *kwanso*. CB-8534: An isolate from *H. fulva* var. *disticha*.

b) ++: Small brown spots and bright yellow stripes. +: Small brown spots with yellow halo. ±: Only small brown spots. —: No symptom.

Table 2. Effect of temperature on the growth of *Aureobasidium microstictum* on PSA medium and MA medium

PSA medium								
Isolate	3	8	17	20	24	28	30	37(C)
CB-8533 ^{a)}	— ^{b)}	+	+++	+++	+++	++	+	—
CB-8535	—	+	+++	+++	+++	++	++	—
CB-8534	—	+	+++	+++	+++	++	++	—

MA medium								
Isolate	3	8	17	20	24	28	30	37(C)
CB-8533	—	+	+++	+++	+++	++	+	—
CB-8535	—	+	+++	+++	+++	++	++	—
CB-8534	—	+	+++	+++	+++	++	+	—

a) CB-8533 and CB-8535 : Isolates from *H. fulva* var. *kwanso*. CB-8534 : An isolate from *H. fulva* var. *disticha*.

b) +++ to + : Vigorous growth to slight growth. — : No growth.

growth, CB-8533 dark brown hyphal growth. On CA, all isolates showed a marked tendency to form yeastlike colonies.

Optimum temperature for growth on media

Growth occurred in the range from 8 to 30 C, while no growth at 3 and 37 C. Optimum growth on PSA was at around 20 to 24 C. Effect of temperatures for the growth on MA were similar to those on PSA (Table 2).

Identification

The morphological characteristics of acervuli, conidiogenous cells and conidia, and restricted host-range of the present fungus indicated that the causal pathogen of *H. fulva* var. *kwanso* seems most closely related to *Aureobasidium microstictum* (Bubák) W. B. Cooke (Table 3). *Aureobasidium microstictum* has been reported to show pathogenicity only on *Hemerocallis* sp. and on *Convallaria majalis*⁴⁾, and our inoculation experiments indicated that the present fungus was also pathogenic on three varieties of *Hemerocallis fulva*. According to the description given by Hermanides-Nijhof³⁾, we identified the present fungus as *Aureobasidium microstictum* (Bubák) W. B. Cooke.

Discussion

In Japan, only rust has been known as a disease of *H. fulva* var. *kwanso*. Therefore, the present disease of *H. fulva* var. *kwanso*, *H. fulva* var. *disticha* and *H. fulva* var. *fulva* was considered as a new disease caused by *A. microstictum*, and a common name, leaf blight, was proposed⁸⁾.

It was reported that *A. microstictum* was isolated from *Hemerocallis* sp. in Netherlands and from *Convallaria majalis* in West Germany⁴⁾. Recently, occurrence of a new disease of *H. fulva* var. *fulva* caused by *Aureobasidium* sp. in Taiwan was reported⁶⁾. The fungus seems to be *A. microstictum*. The present fungus was isolated for the first

Table 3. Comparison of major morphological characteristics of *Aureobasidium microstictum* (Bubák) W. B. Cooke

Fungus		<i>Aureobasidium microstictum</i> ^{a)} (The present fungus)	<i>Aureobasidium microstictum</i> ^{b)} (Authentic fungus)
On host plant	Acervuli	tightly interwoven hyphae	tightly interwoven hyphae
	Conidiogenous cells	clavate to subcylindrical hyaline smooth blastic conidia are formed at the apex distinct scars remain at the apex 30-140×4-10 μ m (76.4×6.6 μ m) ^{c)}	clavate to subcylindrical conidia are formed at the apex indistinct scars remain at the apex 13-33×5.5-11 μ m
	Conidia	ellipsoidal to fusoid hyaline smooth one-celled secondary conidia present 5.5-18×2-4.5 μ m (9.6×3.2 μ m)	ellipsoidal hyaline smooth one-celled secondary conidia present 6-15×2.5-4 μ m
On medium	Hyphae	hyaline smooth brown hyphae present 2-6.5 μ m (4 μ m)	hyaline smooth brown hyphae present 3-4 μ m
	Conidia	ellipsoidal to fusoid hyaline smooth one-celled secondary conidia present 5.5-20×2-5 μ m (9.4×3.7 μ m)	ellipsoidal hyaline smooth one-celled secondary conidia present 5-16×2.5-5 μ m
	Chlamydospores	ellipsoidal brown 8-27×4-15 μ m (17×7.9 μ m)	—
References		M. Yoshikawa and T. Yokoyama (1986)	E. J. Hermanides-Nijhof (1977)

a) Host : *Hemerocallis fulva* var. *kwanso*, Medium : potato-dextrose agar medium (IFO 32065 isolated from *H. fulva* var. *kwanso*)

b) Host : *Convallaria majaris* (type specimen), Medium: malt agar medium (CBS 114.64 isolated from *Hemerocallis* sp.)

c) Figures in parenthesis show mean value.

time in Japan.

Plant pathogenic species of *Aureobasidium* have been assigned to *Kabatiella* in Japan: *K. zeae*, *K. caulivora* and *K. sorghi* have been known on corn, red clover and sorghum, respectively. *Aureobasidium microstictum* was originally described as *K. microsticta* Bubák¹⁾. *Kabatiella* was reduced to a synonym of the genus *Aureobasidium*, and a new combination name, *A. microstictum* (Bubák) W. B. Cooke, was proposed²⁾. We follow the W. B. Cooke's system of classification of *Aureobasidium*.

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和 文 摘 要

吉川正巳・横山竜夫 : *Aureobasidium microstictum* (Bubák) W. B. Cooke によるカンゾウ類葉枯病 (新称)

1985年6月下旬, 京都府綾部市の京都府農業総合研究所薬用植物園で, ヤブカンゾウに褐点~黄色条斑症状株が発生した。本病は11月中旬まで発生が続き, 初め葉の両面に褐色, 小型の斑点を形成し, 後に鮮黄色条斑を呈し, やがて葉先から褐変枯死する。通常, 罹病葉上では病原体は認められなかったが, 温室中で病斑上に密に重り合った菌糸からなる子座と棍棒形~亜円筒形の分生子形成細胞及びその頂端部に楕円形~紡錘形, 無色, 平滑, 単室の分生子を出芽的に形成した。分離菌はヤブカンゾウ, ノカンゾウ, ホンカンゾウに有傷接種で病原性を示した。ノカンゾウにも自然発病が認められ, 分離菌はヤブカンゾウ分離菌と同様の病原性を示した。単胞子分離菌株は PDA 培地上で最初はクリーム色, 酵母状を呈し, 後に白色菌糸状となり, さらに暗褐色~黒色となる。生育温度は 8~30°C, 生育適温は 20~24°C 付近である。分生子は楕円形~紡錘形, 無色, 平滑, 単室で出芽的に形成され, しばしば酵母状の増殖をする。以上の形態的特徴とカンゾウ類に対する病原性から, 本菌を *Aureobasidium microstictum* (Bubák) W. B. Cooke と同定し, 病名として葉枯病を提唱する。

Explanation of plates**Plate I**

Photographs of symptoms naturally occurring on the leaves of *Hemerocallis fulva* var. *kwanso*.

1. Small brown spots.
2. Bright yellow stripes.
3. Symptoms occurred on the lower leaves.
4. Brownings from the leaf tips.

Plate II

Aureobasidium microstictum (Bubák) W. B. Cooke.

On naturally infected *Hemerocallis fulva* var. *kwanso*.

5. Vertical section of acervulus.
6. Apical portions of conidiogenous cells.
7. Conidia and secondary conidia.

On PDA culture.

8. Conidiogenous cells.
9. Conidia and secondary conidia.
10. Chlamydospores.

Plate III

Photomicrographs of naturally occurring acervulus, conidiogenous cells and conidia of *Aureobasidium microstictum* on *Hemerocallis fulva* var. *kwanso*.

11. Vertical section of acervulus.
12. Conidiogenous cells protruding through the acervulus.
13. Conidia.

Photomicrographs of conidiogenous cells, conidia and chlamydospores on PDA culture.

14. Conidiogenous cells and polyblastic conidia.
15. Conidia.
16. Chlamydospores.

(Scale bars=20 μ m)

Plate I



Plate II

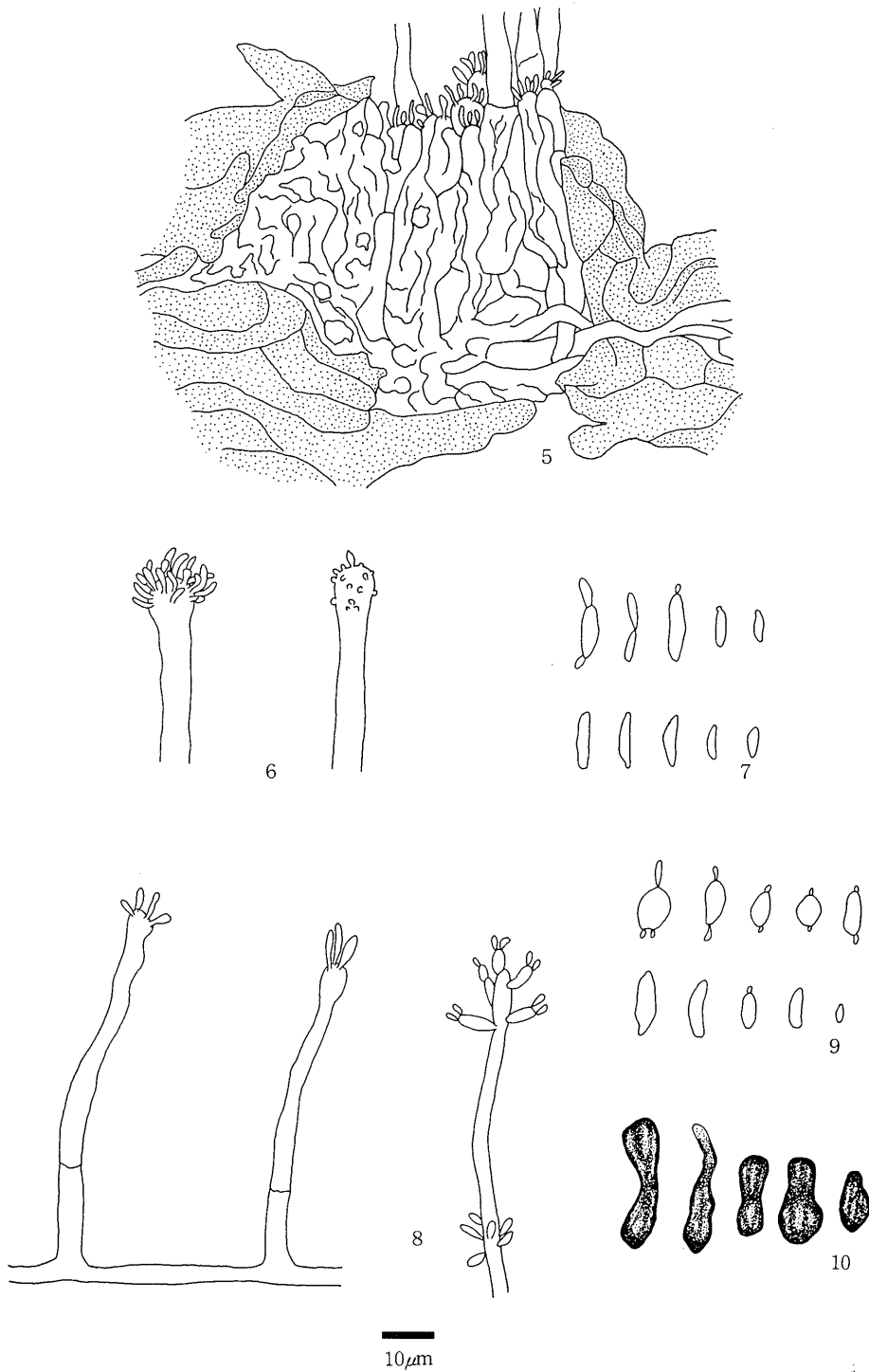


Plate III

