

Brown spot of *Rheum officinale* in Indonesia with special reference to the variation in conidia of the causal fungus

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Brown spot disease of *Rheum officinale*, a traditional medicinal plant in South East Asia, was newly recorded from the highland area of West Java Province. After confirmation of its pathogenicity toward *Rheum officinale* and comparison of its morphology with hitherto known species, the casual fungus was identified as *Ascochyta rhei*. It produces widely varied conidia from unicellular and bacillar conidia (*Phoma*-type), 1-septate oblong conidia (*Ascochyta*-type) to 2–3-septate cylindrical conidia. These conidia were sometimes formed within the same pycnidium. They could germinate easily and produce the similar colonies to each other.

Key Words—*Ascochyta rhei*; Brown spot; conidial variation; medicinal crop; new to Indonesia; *Rheum officinale*.

Introduction

During the survey on diseases of spice and medicinal crops in Indonesia, a severe leaf spot disease on *Rheum officinale* Baill. was found in the Manoko Experimental Garden of BALITTRO, West Java. The stem of the plant is commonly used as a traditional medicine to treat malaria, tropical thrush (sprue), or cough (Kasahara and Hemmi, 1986). It is also used as a vegetable. The plant is cultivated and grows in highland areas of Java. The disease was thought to be new to Indonesia. This paper reports etiological studies of the disease and information on variation of sizes in conidia of the casual fungus. A summary of the results of the study was preliminarily reported and a new disease name in Japanese was proposed (Kobayashi et al., 1993).

Etiological studies

1. Symptoms and damage of the disease (Fig. 3A, B; Fig. 4A, B) Leaf spot are brown, circular, 2–5 mm in diameter, enlarging to form large spots with dark brown, concentric zones. Spots often coalesce to form big, irregular and necrotic patches surrounded with yellowish areas. Finally the necrotic areas become dry rot with many shrinks. On the necrotic areas, pycnidial pustules of the casual fungus are formed as brown to dark brown dots. Damage of the disease seems to be moderate to heavy. The plant and the disease are distributed in highland areas of Java.

2. Morphology of the fungus (Fig. 1; Fig. 3D, E; Fig. 4C–G) Pycnidia first immerse within leaf tissues, then the upper part of a pycnidium breaks through the epidermal layer appearing an ostiole, lenticular to globose, 80–225 μm in diameter, 70–15 μm in height, with thin, brown to dark brown and membranaceous wall, 5–15 μm in thickness; conidiophores or conidiogeous cells are hyaline, short, denticle-like; two types of conidia are produced on the brown spot lesions; one hyaline, unicellular, bacillar, and 6.3–10 \times 2–2.5 μm ; the other hyaline, 1-septate, oblong, and 12.5–17.5 \times 2.5–4.5 μm .

Among three materials from Indonesia, one (TFM: FPH-7253) has only unicellular small-sized conidia, and one (TFM: FPH-7254) has both unicellular and 1-septate conidia. Both types of conidia in the latter material were produced within the same pycnidium. The third one (TFM: FPH-7055) had no pycnidia on the brown spot lesions.

Both types of conidia could easily germinate on water agar (WA) and 2% glucose agar (GA). Germinated conidia developed similar colonies to each other on potato sucrose agar (PSA) slants. Both types of conidia were produced on the colonies isolated from two materials mentioned above.

3. Pathogenicity of the fungus (Fig. 3C) Inoculation tests were carried out at the Manoko Experimental Garden of BALITTRO, located at 1,300 m above sea level, where the host plant grows well. *Rheum* plants could not grow well under the environmental conditions of Bogor, at 250 m above sea level. As inoculum, conidial suspension obtained from PSA slant culture of the fungus was used. This suspension included both of unicellular and 1-septate conidia. Leaves of the potted *Rheum* plants were sprayed with conidial suspensions of the fun-

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gus. Inoculated plants were kept under moist conditions by covering them with polyethylene bag for 3 days. As a control, sterilized water was sprayed onto seedlings.

Initial symptoms appeared 4–6 days after removing polyethylene bags, as light brown small spots. Then the spots increased in number and size, as in the case of naturally infected plants. Fruiting bodies were also produced on the concentric zones of spots. From conidia on artificially infected spots, similar colonies to inoculum were obtained.

4. Mycelial growth and conidial production under different temperatures (Fig. 2; Fig. 3F) Cultural characteristics of the fungus were observed on PSA under different temperatures, at 4, 13, 20, 24 and 30°C. Growth rate

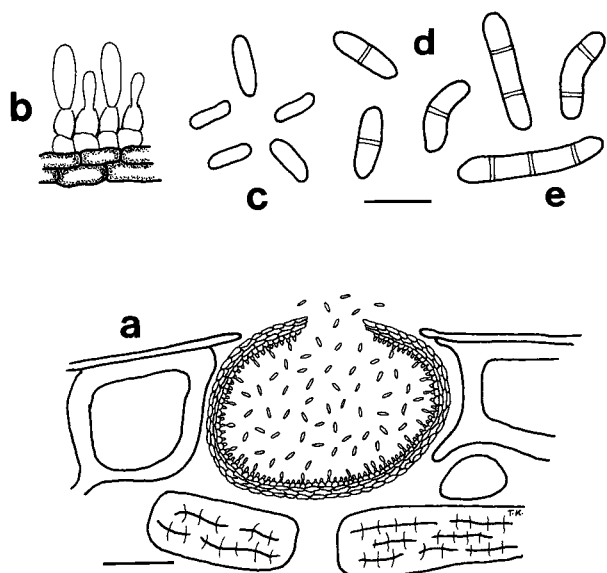


Fig. 1. Brown spot fungus on *Rheum* (*Ascochyta rhei* Ell. et Ev.), having varied types of conidia.

a: Pycnidium, b: A part of pycnidial wall, c: 0-septate (*Phoma*-type) conidia, d: 1-septate (*Ascochyta*-type) conidia, e: 2–3-septate conidia. (a–d: Indonesian specimen TFM: FPH-7254; e: American specimen USO: 372246) (—: a = 50 μm ; b–e = 10 μm)

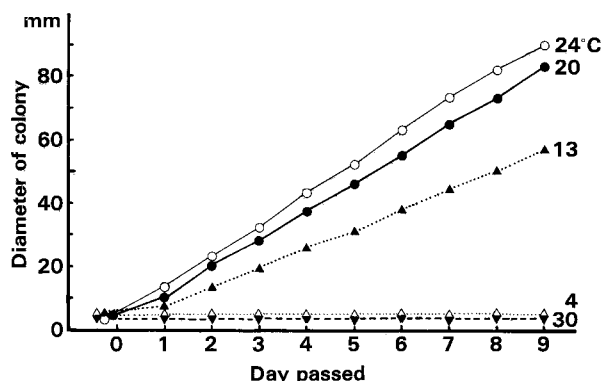


Fig. 2. Mycelial growth of brown spot fungus at different temperatures.

of colonies was measured every day for 9 days. Petri dishes of 9 cm in diameter kept at 24°C were fully covered by the mycelial colonies after 9 days. Thereafter, production of pycnidia was observed up to one month.

As shown in Fig. 2, the fungus could grow under at all tested temperatures, with the optimum at 24°C. Colonies of the fungus are initially dirty white, subsequently dark brown with concentric zones composed of whitish aerial mycelia. No pigmentation was observed. At 20 and 24°C, production of pycnidia started within 3 to 4 days after transplanting. Sticky conidial oozes, pale orange to grayish brown in color, were produced from blackish masses of pycnidia on the central part of colonies. At 13°C the fungus produced pycnidia 7 days after transplanting. Although no growth of colonies was observed at 4°C within 9 days, the fungus could grow colonies of 20 mm in diameter with conidial oozes after one month. At 30°C, no growth of colonies was recorded up to one month. The fact that the fungus could not form colonies at 30°C, is thought to be one of the reasons that the disease has been observed only in areas with a cool climate, where are more than 1,000 m above sea level in Java.

Variation of conidial sizes and identification of the fungus

As mentioned above, the causal fungus produces two types of conidia in size and shape. One is unicellular, hyaline and bacillar conidia, which correspond to the genus *Phoma* Saccardo in the sense of Boerema (1963). They do not belong to the genus *Phyllosticta* Saccardo in the sense of van der Aa (1973), which has globular appendaged conidia and the *Guignardia* teleomorph.

The other form is 1-septate, hyaline and oblong conidia, which are distinct from the unicellular *Phoma*-type conidia in size as shown in Table 1 and Fig. 5. They should belong to the genus *Ascochyta* Libert in the sense of Punithalingam (1979) in their size and shape.

Phoma rheina (Thümen et Boll.) and *Phyllosticta rhei* Ellis et Everhart 1889 non 1891 (Saccardo, 1892) have hitherto been described on *Rheum* plants. As shown in Table 1 and Figs. 5 to 7, *Phoma rheina* is apparently different from the Indonesian fungus in the small size of its conidia (1.5 \times 1 μm). The shape and size of conidia of *Phyllosticta rhei* Ell. et Ev. 1889 non 1891 (5–7 \times 2–2.5 μm) accord with those of Indonesian *Phoma*-type conidia (5–10 \times 2–2.5 μm).

On the other hand, Ellis and Everhart have described other *Phyllosticta* species on *Rheum rhaponticum*, namely, *P. rhei* Ell. et Ev. 1891 non 1889. The size of conidia of this *Phyllosticta* (7–12 \times 3.5–4.5 μm) differs from that of *P. rhei* described in 1889. Ellis and Everhart transferred and redescribed it as *Ascochyta rhei* Ell. et Ev. in 1893. At that time, they changed the description of conidia from unicellular to 1-septate. Besides *Ascochyta rhei*, another *Ascochyta* species has been described from Japan on *Rheum rhaponticum*, namely, *A. rheicola* Sawada (1958). Morphological characteristics of *Ascochyta*-type conidia in the Indonesian fungus having a septum in the middle part (12.5–17.5 \times 2.5–4.5 μm)

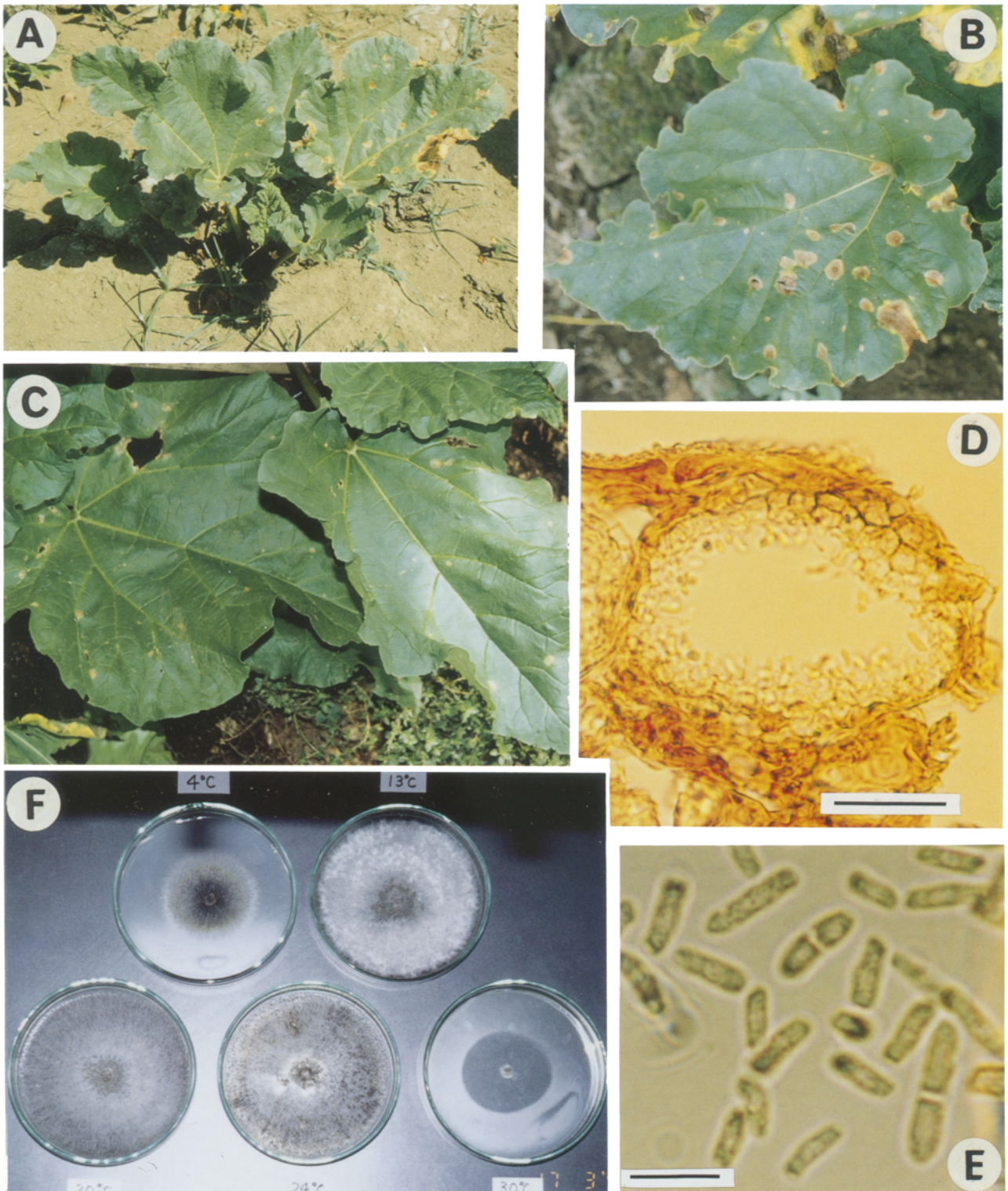


Fig. 3. Brown spot disease and its pathogen of *Rheum officinale*.

A: Infected stock showing brown spots, B: Magnified symptoms, C: Early symptom caused by inoculation, D: Cross-section of pycnidia, E: 0- (*Phoma*-type) and 1-septate (*Ascochyta*-type) conidia, F: Mycelial growth at different temperatures. (—: D=50 μ m; E=10 μ m.)

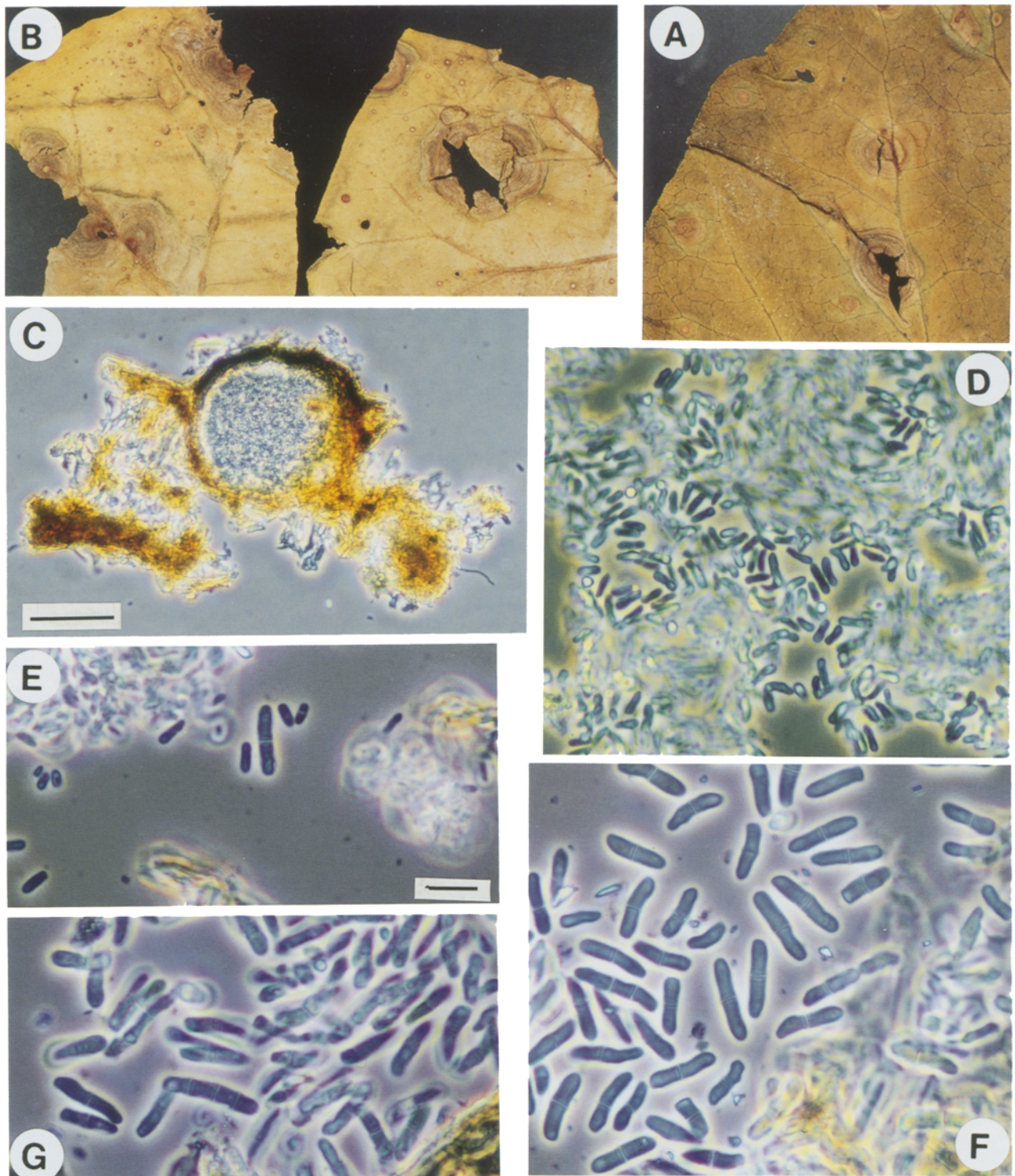


Fig. 4. Brown spot disease on *Rheum* spp. and varied types of conidia.

A: Spot lesions on *Rheum officinale* from Indonesia (TFM: FPH-7254), B: Spot lesions on *R. rhaponticum* from USA (USO: 372254), C: Pycnidium having 0-septate type conidia (USO: 372250), D: 0-septate conidia (TFM: FPH-7253), E: 0- and 1-septate conidia (USO: 372277), F: 1- and 2-septate conidia (UFO: 372246), G: 1-, 2- and 3-septate conidia (USO: 372270). (—: C = 100 μm ; D-G = 10 μm .)

seem to be quite similar to those of *Ascochyta rheicola* (11–18 × 4.5–5 μm) and somewhat similar to those of *A. rhei* (7–12 × 3.5–4.5 μm).

Therefore, 33 specimens of *Ascochyta rhei* and *Phyllosticta rhei* from the National Fungus Collection of U.S.

Department of Agriculture, including 7 specimens from Ellis' Herbarium were examined. Symptoms of leaves on these specimens were identical with those of Indonesian diseased materials (Fig. 4). Results of measurement are shown in Tables 1 to 2 and Figs. 5 to 7.

Table 1. Dimensions of the fungi with 0-septate (*Phoma*), 1-septate (*Ascochyta*) or 2–3-septate conidia on *Rheum* spp. (μm).

Species or materials	0-septate conidia	1-septate conidia	2–3-septate conidia	
<i>Phoma rheina</i> Thüm. et Boll.	1.5 × 1			a)
<i>Phyllosticta rhei</i> Ell. et Ev.	5–7 × 2–2.5			b)
<i>Ascochyta rhei</i> Ell. et Ev.		7–12 × 3.5–4.5		c)
<i>Ascochyta rheicola</i> Sawada		11–18 × 4.5–5		d)
Indonesian material (TFM: FPH-7253)	5–10 × 2–2.5			e)
" " (TFM: FPH-7254)	7.5–10 × 2–2.5	12.5–17.5 × 2.5–4.5		e)
American material (USO: 372246)		12.5–15 × 3.8–5	13.8–20 × 3.8–5	f)
" " (USO: 372250)	7.5–10 × 1.3–2.5			f)
" " (USO: 372251)		11.3–17.5 × 3.8–5		f)
" " (USO: 372252)	8.8–15 × 2–2.5	12.5–17.5 × 2–3		g)
" " (USO: 372253)	7.5–11.3 × 2–2.5	10–13.8 × 2.5–3		f)
" " (USO: 372254)		15–20 × 3.8–5		g)
" " (USO: 372256)		13.8–18.8 × 3.8–5		g)
" " (USO: 372257)	7.5–10 × 2.5–3			g)
" " (USO: 372258)	5–7.5 × 2–2.5	15–20 × 3.8–4.5		g)
" " (USO: 372259)	5–7.5 × 2–2.5	10–15 × 2.5–3		g)
" " (USO: 372260)	5–8.8 × 2	13.8–17.5 × 3–4.5		g)
" " (USO: 372264)	5–10 × 2–2.5	13.8–20 × 3–4.5		g)
" " (USO: 372265)		13.8–17.5 × 3–5		g)
" " (USO: 372269)		10–15 × 2.5–4.5		g)
" " (USO: 372270)		12.5–17.5 × 3–5	16.3–21.3 × 3.8–5	g)
" " (USO: 372271)		12.5–15 × 3–4.5		g)
" " (USO: 372272)		15–17.5 × 3–4.5		g)
" " (USO: 372274)	5–10 × 2–2.5			g)
" " (USO: 372276)		13.8–17.5 × 3.8–4.5	16.3–20 × 3.8–5	g)
" " (USO: 372277)	5–10 × 2–2.5	11.3–15 × 3–4.5		g)
" " (USO: 372279)	6.3–10 × 2–3	10–16.3 × 3–4.5		g)
" " (USO: 372280)		12.5–17.5 × 3–4.5	16.3–20 × 3.8–4.5	g)
" " (USO: 372281)	5–7.5 × 2–2.5			g)
" " (USO: 374557)	5–10 × 2–2.5			f)
" " (USO: 374558)		13.8–20 × 2.5–3.8		g)

a) Saccardo 1892, on *Rhem rhaponticum*; b) Ellis et Everhart 1889, on *R. officinale*; c) Ellis et Everhart 1891 and 1893, on *R. rhaponticum*; d) Sawada 1958, on *R. rhaponticum*; e) the authors, on *R. officinale*; f) specimen deposited at National Fungus Collections, U.S. Department of Agriculture, on *R. sp.*; g) ditto, on *R. rhaponticum*. a)–d): from the original description. e)–g): dimensions measured by the authors.

Table 2. Specimens of brown spot disease of *Rheum* spp. differentiating by the type of conidia^{a)}.

0-septate conidia	Both 0- and 1-septate conidia	1-septate conidia	Both 1- and 2–3-septate conidia	No pycnidia
5	10	8	4	10
A = 15 (37%)		B = 22 (54%)		C = 4 (10%)

^{a)} 3 Indonesian and 34 American specimens.

A: Specimens having 0-septate conidia, B: Specimens having 1-septate conidia, C: Specimens having 2–3-septate conidia.

As observed in the Indonesian specimens, two types of conidia, non-septate and septate conidia, were also recognized among the American specimens, not only on the same leaf spot but also in the same pycnidium. As shown in Fig. 5, average sizes of unicellular *Phoma*-type conidia in each specimen are closely grouped and clearly distinct from those of septate conidia. Morphological characteristics of these conidia are identical with those of *Phyllosticta rhei* Ell. et Ev. 1889 non 1891, as in the case of the Indonesia fungus.

As shown in Table 2, in 4 American specimens, 2-3-septate conidia were observed besides common 1-septate conidia. In the concept of modern systematics of Deuteromycotina (Sutton, 1980), no adaptable genus has been found for 2-3-septate conidia, though in Saccardo's sense they are included in the genus *Stagonospora* Saccardo. General morphological characters of the Indonesian *Ascochyta*-type conidia accord fully with those of the American 1-septate conidia and also with those of *Ascochyta rheiicola* described by Sawada as shown in Table 1 and Fig. 5. No species of *Stagonospora* has been described on *Rheum* plants. In our study, as shown in Table 2, 54% of specimens had *Ascochyta*-type conidia, 37% *Phoma*-type, but only 10% 2-3-septate conidia.

Considering the above facts, we are inclined to ac-

cept the species name *Ascochyta rhei* Ellis et Everhart for the fungus on *Rheum* spp., which has wide variation of size and shape in its conidia and causes serious brown spot disease. *Phyllosticta rhei* Ellis et Everhart and *Ascochyta rheiicola* Sawada were treated as synonyms of

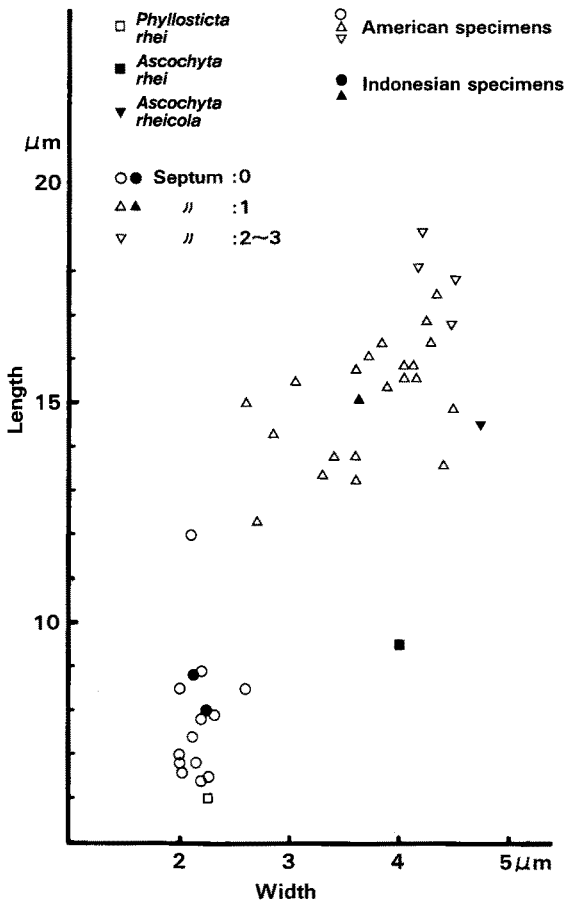


Fig. 5. Average size of conidia of the brown spot fungus on *Rheum* spp.

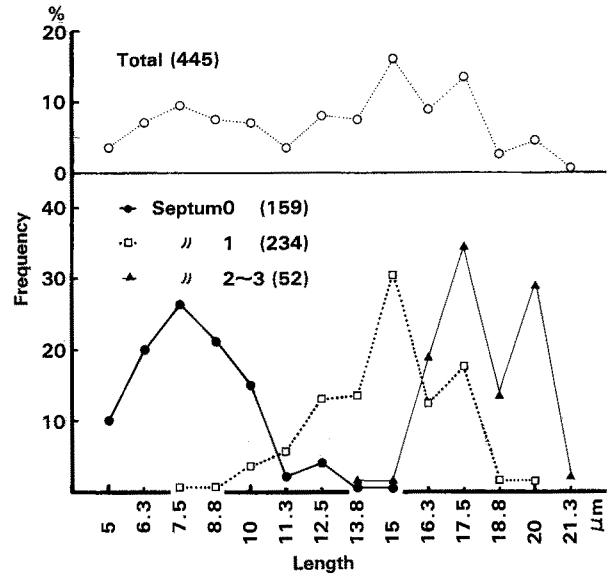


Fig. 6. Length variation of conidia having different numbers of septa (number of conidia measured).

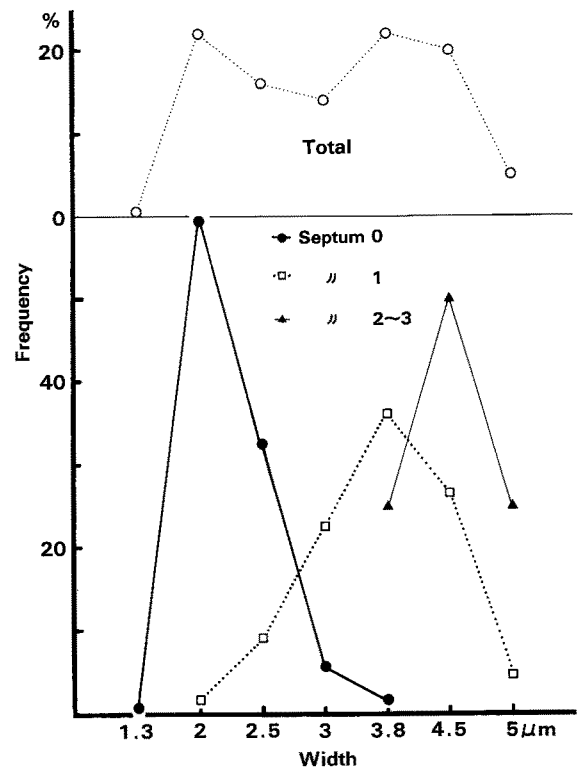


Fig. 7. Width variation of conidia having different numbers of septa (number of measured conidia is the same as in Fig. 6).

Ascochyta rhei. A revised description of *Ascochyta rhei* is as follows.

***Ascochyta rhei* Ellis et Everhart**, Proc. Acad. Philad. 1893: 160 (1893); Saccardo, Syll. Fung. 9: 525, 1892.

Synonym: *Phyllosticta rhei* Ellis et Everhart 1889 non 1891, Journ. Mycol. 1889: 145 (1889); Saccardo, Syll. Fung. 9: 134, 1892.

Phyllosticta rhei Ellis et Everhart 1891 non 1889, Proc. Acad. Philad. 1891: 177 (1891); Saccardo, Syll. Fung. 9: 135, 1892.

Ascochyta rheiicola Sawada, Bull. Gov. For. Exp. Sta. 105: 53, 1958.

Leaf spots are brown, circular with concentric zones, 10–30 mm in diam., often coalesce to form large leaf blight. Pycnidia densely scattered on the spots, globular, dark brown, 80–225 μm in diam., 70–150 μm in height. Three types of phialidic conidia are produced. Unicellular *Phoma*-type conidia are hyaline, bacillar and 5–11.3 \times 1.3–3 μm , mostly 6.5–10 \times 2–2.5 μm . One-septate *Ascochyta*-type conidia are hyaline, oblong or somewhat irregular in shape, and 7.5–20 \times 2–5 μm , mostly 12.5–17.5 \times 3–4.5 μm . Two- to three-septate conidia are hyaline, oblong to cylindrical, often irregularly curved, and 13.8–21.3 \times 3.8–5 μm , mostly 16.3–20 \times 3.8–5 μm .

Habitat: on living leaves of *Rheum officinale* Baill. ("kelembak" in Indonesian), *R. rhaponticum* L. ("Marubaidaiou" in Japanese) and *R. sp.*

Specimen examined: *Rheum officinale*—Manoko Experimental Garden, BALITTRO, Lembang, West Java, Indonesia, 17 Nov., 1990, by Takao Kobayashi (TK), S. R. Djiwanti (SRD) and Masaomi Oniki (MO) (TFM*: FPH-7253); 2 Jul., 1991, by TK, SRD and MO (TFM: FPH-7254); 25 Mar., 1992, by TK and SRD (TFM: FPH-7055). *Rheum rhaponticum*—Syracuse, New York, USA Jul. 1892, by F. C. Stevens (USO: 372260** labelled as *Phyllosticta rhei* E. et E.); Syracuse, New York, USA, Jul. 1892, by F. C. Stevens (USO: 372262 as *Phyllosticta rhei* E. et E.); Racine, Wisconsin, USA, 28 Aug., 1897, by E. F. Smith (USO: 372265 as *Phyllosticta rhei* E. et E.); Smith-ville, Oregon, USA, 13 Aug., 1898, by C. Z. Yoder (USO: 372264 as *Phyllosticta rhei* E. et E.); Westville, Connecticut, USA, 14 Aug., 1902, by S. P. Boll (USO: 354558 as *Phyllosticta rhei* E. et E.); New Heaven, Connecticut, USA, 29 Jul., 1902, by C. P. Clinton (USO: 372257 as *Ascochyta rhei* E. et E.); New Heaven, Connecticut, USA, 29 Ju., 1902, by S. P. Boll (USO: 372270 as *Phyllosticta rhei* E. et E.); Moscow, West Virginia, USA, Jun. 1902, by J. k. Blotuer (USO: 372280 as *Ascochyta rhei* E. et E.); Kerney, Nebraska,

USA, 31 Ju., 1902, by J. M. Bates, (USO: 372282 as *Ascochyta rhei* E. et E.); Felton, Delaware, USA, 30 Ju., 1906, by H. S. Jackson (USO: 372258 as *Ascochyta rhei* E. et E.); Felton, Delaware, USA, 30 Ju., 1906, by H. S. Jackson (USA: 372274 as *Ascochyta rhei* E. et E.); Norfolk, Virginia, USA, Jul. 1909, by B. L. Waston (USO: 372277 as *Ascochyta rhei* E. et E.); Madison, Wisconsin, USA, 20 Oct., 1912, by J. McMurphy (USO: 372255 as *Ascochyta rhei* E. et E.); Beatrice, Nebraska, USA, 7 Jun., 1916, by G. H. Hedgcock (USO: 372266 as *Ascochyta rhei* E. et E.); Admiral, Maryland, USA, 31 May, 1917, by C. H. S. (USO: 372245 as *Ascochyta rhei* E. et E.); Woodham, Long Island, USA, 27 Aug., 1922, by C. A. Schwarze (USO: 372275 as *Ascochyta rhei* E. et E.); Carbon, Pennsylvania, USA, 5 Oct., 1920, by J. R. Weir (USO: 372252 as *Ascochyta rhei* E. et E.); Wiggins, Mississippi, USA, 2 Aug., 1920, by L. E. Miles (USO: 372276 as *Ascochyta rhei* E. et E.); New Brunswick, New Jersey, USA, 6 Aug., 1923 (USO: 372259 as *Ascochyta rhei* E. et E.); Stratford, Connecticut, USA, 22 Jul., 1927, by C. F. Clinton (USO: 372271 as *Ascochyta rhei* E. et E.); Parkersburg, West Virginia, USA, 8 Oct., 1928, by W. A. Archer (USO: 3722254 as *Ascochyta rhei* E. et E.); Thomaston, Connecticut, USA, 24 Aug., 1928, by E. M. Stddard (USO: 372269 as *Ascochyta rhei* E. et E.); Morgantown, West Virginia, USA, 9 Dec., 1928, by W. A. Archer (USO: 372279 as *Ascochyta rhei* E. et E.); Bantam, Connecticut, USA, 10 Jun., 1934, by McDonnell (USO: 372272 as *Ascochyta rhei* E. et E.); Madison, Wisconsin, USA, 21 Aug., 1954, by H. C. Greene (USO: 372256 as *Ascochyta rhei* E. et E.).

On *Rheum sp.*—Racine, Wisconsin, USA, Aug, 1877, by E. F. Smith (USO: 372246 as *Ascochyta rhei* E. et E. = *Phyllosticta rhei* E. et E.); Racine, Wisconsin, USA, Aug. 1877, by E. F. Smith (USO: 372247 as *Ascochyta rhei* E. et E. = *Phyllosticta rhei* E. et E.); Martic Forge, Pennsylvania, USA, 28 Jul., 1917, by J. F. Collins (USO: 354557 as *Phyllosticta rhei* E. et E.); Cape Girardeau, Montana, USA, 31 Jul., 1926, by W. A. Archer (USO: 372250 as *Ascochyta rhei* E. et E.); Shenandoah, Iowa, USA, 2 Jul., 1927, by W. A. Archer (USO: 372251 as *Ascochyta rhei* E. et E.).

The following specimens have no pycnidium on their leaf spots: on *Rheum rhaponticum* L.—16 Sept., 1892, Barthoromew (USO: 372261 as *Ascochyta rhei* E. et E. = *Phyllosticta rhei* E. et E.); Admiral, Maryland, USA, 31 May, 1919, by C. H. S. (USO: 372283 as *Ascochyta rhei* E. et E.); Brookings, South Dakota, USA, 9 Jul., 1924, by A. T. Evans (USO: 372267 as *Ascochyta rhei* E. et E.); Butler, Colorado, USA, 8 Sept., 1926, by W. A. Archer (USO: 372268 as *Ascochyta rhei* E. et E.); Hamden, Connecticut, USA, 21 Oct., 1931, by A. D. McDonnell (USO: 372273 as *Ascochyta rhei* E. et E.); Moscow, West Virginia, USA, 5 Sept., 1932, by J. K. Bletues (USO: 372278 as *Ascochyta rhei* E. et E.); Bantam, Connecticut, USA, 10 Jun., 1934, by D. McDonnell (USO: 372281 as *Ascochyta rhei* E. et E.); Plymouth, Indiana, USA, by E. A. Osner (USO: 372263 as *Ascochyta rhei* E. et E.). on *Rheum sp.*—Bet Summer and Orting, Washington, USA, 8 Oct., 1930, by W. H. Wheeler (USO:

*Deposited at the Herbarium of Forest pathology and mycology, Forestry and Forest Products Research Institute (TFM), Kukisaki, Ibaraki 305, Japan.

**Deposited at the U.S. National Fungus Collections, Systematic Boptany and Mycology Laboratory, Agricultural Research Service, U.S. Department of Agriculture (BPI), Bldg. 011A, Rm. 304, Beltsville, Maryland 20705-2350, USA.

372249 as *Ascochyta rhei* E. et E. and *Cladosporium* sp.); Plymouth, Indiana, by G. A. Osner (USO: 372248 as *Ascochyta rhei* E. et E.).

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