Typhula japonica n. sp. Isolated from Decayed Leaves of the Rape Plant

By

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(With 1 Plate and 3 Text-figures)

Up to the present, one of the most serious damages to the rape crop in Japan has been attributed to the sclerotium disease caused by *Sclerotinia Libertiana* FUCK., which was studied by many investigators in former days from both mycological and prophylactic points of view. In Northern Japan where rape plants are forced to grow under the snow, it became apparent recently that another sclerotium disease is very destructive to this crop. After the thawing of snow in the early spring it is frequently seen that the rape plants which had made a considerable growth in the previous autumn were severely attacked by a rot disease and their outer lower leaves entirely killed and decayed, leaving merely a few small inner leaves healthy. It reminds one of the so called "winter rot" of cereals and forage grasses caused by *Typhula* spp. under the snow. The injury appears, indeed, to be much more serious than the disease caused by *Sclerotinia Libertiana*. After a close examination of the seriously affected rape plants in the fields the attention of the writer was called to 2 or 3 kinds of sclerotia which were in constant association with the disease. It became evident later that one of them should be considered as a new species belonging to the genus *Typhula*.

The present paper was written to report some morphological and cultural characters of this fungus. Investigations of its pathogenicity are now in progress as well as of the other kind of sclerotia collected in the same fields.

I. Morphological characters of the present fungus

Sclerotia van dyke-brown or chestnut-brown, free on surface of substrate, always single, spherical to somewhat flattened, convex on top, flat or concave below, 0.8–2.0 mm. diam.; rind dark-brown, 10–12 μ thick, composed of a rough, gelatinous layer on the outer walls of large, irregular, peripheral cells; medulla

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prosoplectenchymatous, center of loosely interwoven hyphae. Sporophores stipitate, erect, straight or slightly curved, simple or branched, one or more arising from each sclerotium, 17–40 mm. tall, white; clavula lanceolate or long-

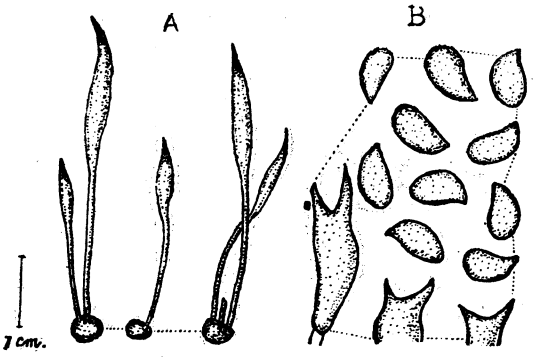


Fig. 1. A. (Natural size) Sporophores arising from sclerotia embedded in the sand.
B. (×620) Basidia and basidiospores.

fusiform, straight to slightly curved, tapering at the apex, 4–20 mm. long, 1–3 mm. broad, white, apex sterile and greyish; stipe distinct in size from clavula, erect or curved, 13–20 mm. long, 0.5–1 mm. broad; basidia elongate, two-spored, ca. 25.3 μ long and 6.3 μ wide; basidiospores pip-shaped, flattened on one side, hyaline, smooth, 11.5–13.8 × 4.6–6.9 μ, average 12.6 × 5.7 μ.

The present fungus somewhat resembles *Typhula variabilis* RIESS from which it differs by its smaller sclerotia and the lack of enlarged cells between the medulla and the rind of the sclerotium, and also by its greyish tinge at the apex of the white sporophores.

The sporophores are produced in the autumn, arising from the sclerotia. When embedded in the sand the sclerotia frequently produced sterile, greyish-brown, filiform and branched sporophores instead of the usual clavate and fertile ones.

Description of the present fungus

Typhula japonica TERUI, sp. nov.

Sclerotiis badiis v. castaneis, superficiis, semper simplicibus, subglobosis, infra planis v. concavis, supra convexis, 0.8–2.0 mm. crassis, 1.4–4.1 mm. diam.; cortice aureo-flavo, 10–12 μ crasso, composito e gelatinosa strue in exterioribus

muris cellarum peripheralium irregulararum detorquearum; medulla omnio prosoplectenchymata, centro solido, hyphis laxe complexis. Sporophoris stipitatis, erectis v. leviter curvis, simplicibus v. raro ramosis, una v. pluribus ex uno sclerotio, 17–40 mm. altis, albis; clavula lanceolata v. longe fusiformi, recta v. leviter curva, 4–20 mm. longa, 1–3 mm. lata, apice sterili et glauca; stipite distincto, recto v. curvo, 13–20 mm. longo, 0.5–1 mm. diam.; basidiis elongatis, bisporis, circ. 25.3 μ longis et 6.3 μ crassis; basidiosporis ovatis v. ellipsoideis, ventricosis, lateraliter apiculatis, hyalinis, levibus, 11.5–13.8 × 4.6–6 9 μ, modus 12.6–5.7 μ.

Hab. in hibernatis caudicibus petiolis et laminis foliorum *Brassicae campestris*. Honshu: Prov. Mutsu, Kuroishi (M. TERUI, 1936). Hokkaido: Prov. Ishikari, Sapporo (M. TERUI, 1940).

Jap. name. *Shiro-gamanohotake* (n. n.).

II. Cultural characters of *Typhula japonica*

A. Material and method

The sclerotia of the fungus used in the present studies were collected at Kuroishi, Prov. Mutsu, northern Japan, 1936. The pure culture of this fungus was obtained by the following method. The sclerotia of the fungus were sterilized with a 0.1 per cent aqueous solution of mercuric chloride for about five minutes, washed repeatedly with sterile distilled water, and then each sclerotium was cut into two or three parts with a sterilized scalpel. The small pieces of sclerotia thus obtained were placed on apricot agar plate and incubated at 13°–15°C.

In 7 to 10 days, mycelia grew from the sclerotia. Fragments of the hyphae were transferred to various agar media. Inoculations were made on the centers of agar plates from the stock cultures on an apricot agar slant and the cultures were placed in an incubator at the temperature of 13°–15°C.

B. Results of culture studies

a. Cultural characters on various media

The cultures on 6 different kinds of agar media were incubated at 13°–15°C for 35 days. The results of observations are shown in the following tables.

Table 1. Cultural characters of the fungus on various media.

Duration of culture	7 days	9 days	13 days	14 days	16 days	19 days		22 days	35 days
Fungous growth / Culture media	Mycelial growth	Production of sclerotia				Mycelial growth	Production of sclerotia	Colour of sclerotia	Appearance of sclerotia
Apricot agar	+	−	−	+	+	+	++	Yellowish brown	Rough
Potato agar	++	−	−	+	++*	++	+++++	Yellowish brown	Very rough
Soy agar	+	−	−	−	+	++++	++	Greyish white	Very dense
Onion agar	+	+	+	++	+++	+	+++++	Blackish brown	Dense
Rape agar	++	−	+	++	+++*	++	++++	Orange yellow	Rough
Asparagin agar	+	−	−	+	+	++	+++	Yellowish brown	Rough

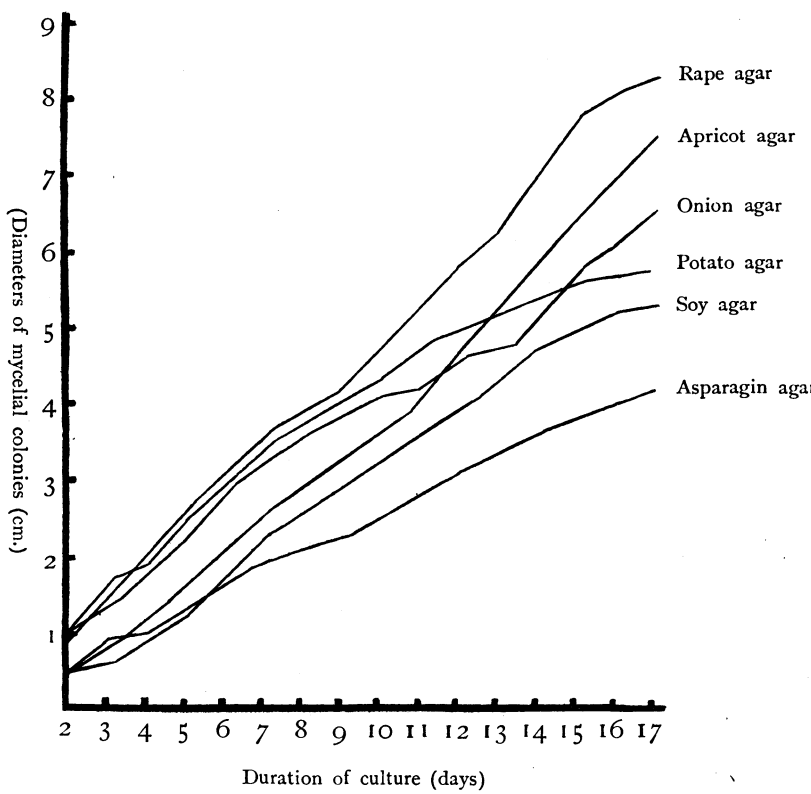
*Sterile sporophores were produced from the sclerotia.

Table 2. Measurements of diameters of mycelial colonies on various culture media.

Duration of culture / Culture media	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days	17 days
	(cm.)																
Apricot agar	0	0.46	0.76	1.13	1.56	2.06	2.50	2.90	3.23	3.53	9.36	4.56	5.13	5.73	6.40	6.93	7.46
Potato agar	0	1.00	1.50	1.86	2.50	2.93	3.33	3.70	4.00	4.33	4.70	4.96	5.16	5.33	5.50	5.63	5.73
Soy agar	0	0.43	0.56	0.80	1.16	1.63	2.13	2.46	2.80	3.20	3.56	3.83	4.16	4.63	4.90	5.13	5.26
Onion agar	0	0.96	1.36	1.73	2.36	2.83	3.20	3.53	3.73	4.03	4.13	4.53	4.66	5.03	5.70	6.06	6.50
Rape agar	0	0.86	1.46	2.00	2.53	3.03	3.50	3.86	4.16	4.66	5.20	5.80	6.23	6.96	7.66	8.00	8.20
Asparagin agar	0	0.43	0.82	0.96	1.30	1.53	1.86	2.03	2.16	2.41	2.62	3.03	3.26	3.52	3.70	3.96	4.13

As shown in the above tables, the medium prepared with the decoction of the leaves of rape plants was the most favorable for the devolment of the fungus. Potato agar, onion agar and apricot agar followed it in descending order. On asparagin agar the growth was very scanty. The formation of sclerotia was the most rapid on the onion agar and they were produced in 9 days. At first the sclerotia were white, scattering on the surface of media, but later they turned dark-brown in colour, and their number increased in the lapse of culture, often gathering into masses of sclerotia. On soy agar, the formation of sclerotia was retarded and white sclerotia began to appear in 16 days. The characters of the sclerotia produced on soy agar were the same as those on the other media. The rates of growth of mycelial colonies are shown in the following figure.

Fig. 2. Graph showing rates of mycelial growth on various culture media.



b. Relation of temperatures to the growth of the fungus

In this experiment, the rape decoction agar was used as the culture medium, and the cultures were placed in incubators at the varying temperatures of 25°–26°C, 18°–20°C, 13°–15°C, 8°–10°C and (−2)°–3°C, respectively.

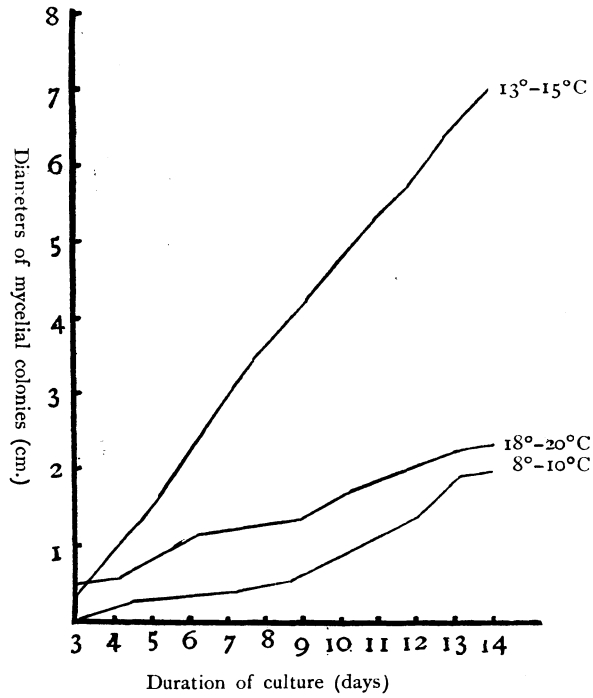
The diameters of mycelial colonies were measured daily for two week after inoculations. The results are shown in the following table.

Table 3. Diameters of mycelial colonies developed at various temperatures.

Duration of culture / Temperature	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
	(cm.)													
25°–26°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18°–20°C	0	0	0.40	0.55	0.80	1.10	1.13	1.16	1.33	1.63	1.80	2.00	2.18	2.26
13°–15°C	0	0	0.38	0.91	1.46	2.26	2.90	3.53	4.06	4.70	5.30	5.76	6.56	7.00
8°–10°C	0	0	0	0.11	0.18	0.28	0.36	0.47	0.65	0.92	1.13	1.37	1.82	1.93
(−2)°–3°C	0	0	0	0	0	0	0	0	0	Scanty growth	Scanty growth	Scanty growth	Scanty growth	Scanty growth

As shown in the above table, the mycelial growth was the most vigorous at 13°–15°C. The minimum and the maximum temperatures for the growth were about −2°C and 20°C, respectively. No mycelial growth was recognized at 25°–26°C throughout this experiment. At 18°–20°C, 13°–15°C and 8°–10°C the hyphae developed in the media were more or less abundant, while the aerial hyphae were produced only scantily at central parts of the mycelial colonies. The rates of the mycelial growth are shown in the following figure.

Fig. 3. Graph showing the rates of mycelial growth at various temperatures.



The so-called winter rots of the winter cereals and forage grasses caused by the fungi belonging to the genus *Typhula* in northern parts of Japan, Europe and North America, have been studied and discussed by many authors. According to them, the pathogenicity of these fungi appears to be beyond doubt.

As to winter crops other than the gramineous ones, BRIZI (1906), RAMBOUSEK (1923), NEUWIRTH (1924) and BENSAUDE (1926) reported that a very destructive disease of sugar beets was caused by *Typhula variabilis* RIESS. VOGLINO (1929) and SCHMIDT (1933) demonstrated the pathogenicity of this fungus on potatoes and asparagus rhizomes in addition to sugar beets.

More recently, REMSBERG (1940) succeeded in the infection of the fungus on stored celery besides beets, potatoes, and asparagus rhizomes.

In our country IMAI (5) reported on *Typhula ishikariensis* IMAI at the Annual Meeting of the Phytopathological Society of Japan, in 1931. He had collected the sclerotia of this fungus from rotten leaves and petioles of red clover and rape plants and suggested that the fungus possibly causes an out-

break of the winter rot disease of these crops under certain environmental conditions.

In regard to the fungus under consideration, the symptoms appeared on the affected plants and the constant association of the sclerotia of the fungus on decayed tissues as well as its physiological characters observed in the cultures led the writer to presume that the present fungus possibly causes the "winter rot" of rape plants under the snow-covering, though its actual pathogenicity to the plants has not yet been experimentally demonstrated.

III. Summary

The present paper deals with a new species of *Typhula* arising from the sclerotia collected from decayed leaves of rape plants in the early spring in northern Japan, with special reference to its cultural characters.

The fungus was named *Typhula japonica* TERUI and a Latin description was given to it.

Out of 6 different kinds of culture media used, the rape decoction agar was the most favorable for the mycelial growth. On asparagin agar, its growth was very scanty.

When the fungus was cultured on rape decoction agar at (−2)°−3°C, 8°−10°C, 13°−15°C, 18°−20°C and 25°−26°C, the mycelial growth was most vigorous at 13°−15°C. The minimum and maximum temperatures for its growth were around (−2)°C and 20°C respectively.

Based upon the evidences of observation, the writer has presumed that the present fungus possibly causes the "winter rot" of rape plants under the snow-covering in the northern parts of Japan.

Finally the writer wishes to express his sincere gratitude to Profs. S. ITO and Y. TOCHINAI under whose directions this work was carried out and also to Dr. T. FUKUSHI for his valuable suggestions and criticisms. He is also indebted to Dr. S. IMAI for his kind advices in the identification of the fungus.

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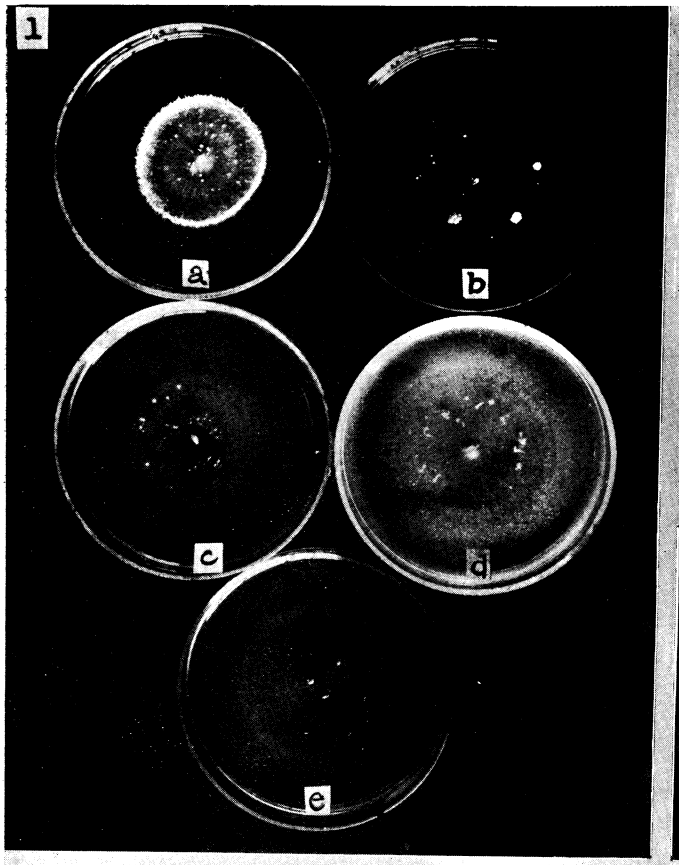
Explanation of plate I

Fig. 1. The fungus on various media after 3 weeks' culture at 8°–10°C.
a. soy agar. b. rape agar. c. onion agar. d. potato agar. e. apricot agar.
Fig. 2. Imperfect fructifications produced from sclerotia which were sown on moist sand in a pot.
Fig. 3. Surface view of a sclerotium, showing the irregular thick-walled cells.
Fig. 4. The fungus on the onion decoction agar after 80 days' culture at 8°–10°C.
Fig. 5. Section of a sclerotium, showing the prosoplectenchymatous medulla.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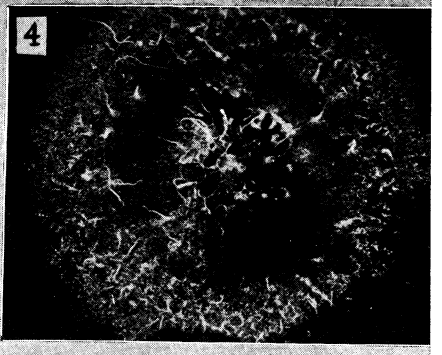
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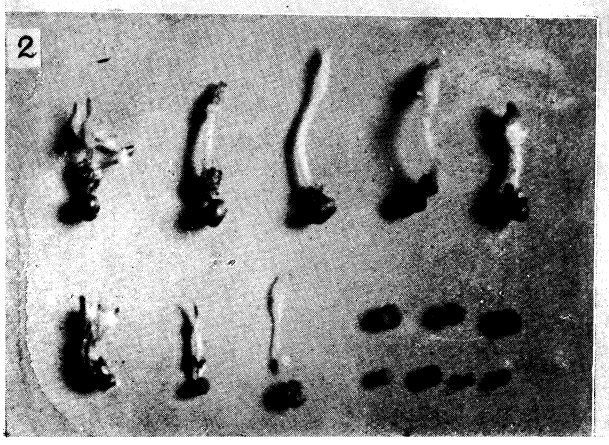
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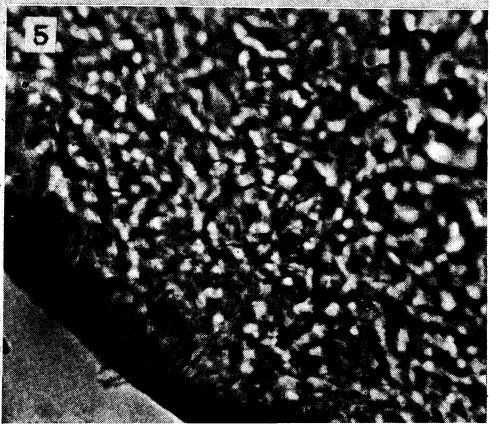
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