# Taxonomic Notes on *Chloroscyphae* causing Needle Blight of Japanese Conifers.

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## Introduction

In 1927, KITAJIMA<sup>11)</sup> reported the occurrence of severe needle blight of Japanese cedar, *Cryptomeria japonica* D. Don, in several forest stands, caused by a species of Discomycete on which he did not determined its systematic position. Later, Tocashi *et al.*<sup>28)20)</sup> found the same disease in the course of a survey on the plant diseases in Iwate Prefecture, the Tohoku district. They identified its causal fungus as a species of the genus *Mollisia*. In 1950, SAWADA<sup>20)</sup> collected a Discomycete which was regarded as the same needle blight as recorded by KITAJIMA (l. c.) and Tocashi *et al.* (l. c.), and he described it as a new species, *Mollisia cryptomeriae* SAWADA. Shortly after, he<sup>21)</sup> added two new species to this genus; one is *Mollisia chamaecyparidis* SAWADA causing the needle blight of *Chamaecyparis pisifera* ENDL. and the other is *Mollisia thujopsidis* SAWADA causing the needle blight of *Thujopsis dolabrata* var. *hondai* MAKINO and *Thuja standishii* CARR. Consequently, three *Mollisiae* have been known as the causal organisms of needle blights among Japanese foresters.

Concerning the genus name *Mollisia* accepted by SAWADA for the blight fungi, a questions has arisen from the view of recent literature on the classification of Discomycetes. Judging from this literature, these fungi probably belong to either the genus *Kriegeria* or the genus *Chloroscypha*. Moreover, several species of *Kriegeria* and *Chloroscypha* have been known from North America and Europe as the causal organisms of needle blight on several kinds of conifers. For this reason, many materials of the needle blight fungi were collected from various localities throughout Japan, and restudies on their identification were carried out by the author. In this paper, results obtained from these restudies will be reported. Important characteristics, being available to differentiate the Japanese needle blight fungi, were preliminarily reported<sup>12</sup>.

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#### Morphology of the needle blight fungi

In the modern classification of Discomycetes<sup>3)7)14)25)</sup>, the structure of apothecia and reaction

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of ascus and ascospore to several reagents, such as MELZER, KOH, cotton blue, etc., are considered to be more important than the size and colour of apothecia as aids to classifying species, genus, family or order. Then, the morphologic characters of the needle blight fungi which had been identified as the members of the genus *Mollisia*, were examined in detail on the materials which were collected newly and deposited in the herbarium of the Station. According to the author's examination and the description given by SAWADA, general features of these needle blight fungi are as follows:

Apothecium on blighted or self-prunned dead needle, black and turbinate under dry condition, cup- to disc-shaped and yellowish green to pale olive under moist condition, about 0.5 mm in diameter, short-stipitate. Ectal excipulum olivish, divided into two layers, inner layer composed of dense slender hyphae arranged parallel upwards, and outer layer composed of loose hyphae and filled with gelatinous substance between them (Plates 2:C, 3:C, D;Figs. 7:B, 8:B, 9:B). Epithecium pale olive, composed of small angular cells and coloured tip of paraphyses (Figs. 7:B, 8:B, 9:B). Hymenium formed with asci and paraphyses in a row (Plates. 2:C, 3:C, D). Hypothecium and medullary excipulum a little (Figs. 7:A, 8:A, 9:A). Ascus hyaline, cylindric to clavate, containing eight ascospores in a single or two rows (Plates 2:D, G, 3:E, F;Figs. 7:C, 8:C, 9:C). Ascospore hyaline to pale yellowish green, non-septate, globular, elliptic, fusoid or boat-shaped (Figs. 7:E, 8:E, 9:E). Paraphysis hyaline, slender, usually branched, swollen and olivish-coloured at the tip (Figs. 7:D, 8:D, 9:D).

# Identification of the genus

TOGASHI *et al.*<sup>28/29)</sup> and SAWADA<sup>20/21)</sup> identified their needle blight fungi as the species of the genus *Mollisia*, probably referring to the classification of CLEMENTS and SHEAR<sup>4)</sup>. However, these fungi having the above-mentioned morphologic characteristics would belong to a genus other than *Mollisia*. According to the recent systematists<sup>307)8)14)25), they belong in the genus *Kriegeria* known commonly in North America or the genus *Chloroscypha* used generally in Europe.</sup>

In 1931, SEAVER<sup>22)</sup> erected the genus *Chloroscypha* based on a fungus collected on *Thuja* plicata D. Don in the United States of America. At that time, the other three fungi were also treated in this genus by SEAVER. Then, a few years later, he<sup>23)</sup> added a species to the genus. However, SEAVER<sup>24)</sup> rejected the generic name *Chloroscypha* in 1943 and adopted *Kriegeria* instead. According to him, general features of the genus *Chloroscypha* quite agree with those of *Ombrophila*? *kriegeriana* described by RABENHORST<sup>16)</sup>. In the notes of the description, RABENHORST stated that "In Bezug auf die Gattung muss ich bemerken, dass ich den Pilz nur vorlaüfig seiner tremellenartigen Beschaffenheit halber zu *Ombrophila* gestellt habe, es wird besser sein, ein besonderes genus zu bilden, zu dem ich den Namen *Kriegeria* vorschlage und die Species dann mit *olivacea* bezeichne". SEAVER<sup>24)</sup> estimated this note as justifiable and treated *Chloroscypha* as a synonym of *Kriegeria* RABENHORST. At the time when the genus *Kriegeria* was accepted by SEAVER, he considered as unnecessary the alternation of species name proposed by RABENHORST, and gave the name *Kriegeria kriegeriana* (RABENH.) SEAVER for the type species. "Dictionary of fungi"<sup>2)</sup> accepted SEAVER's opinion.

On the other hand, DENNIS<sup>5)6)</sup> and TERRIER<sup>27)</sup> did not accept SEAVER's alternation of the generic name from *Chloroscypha* to *Kriegeria*. According to DENNIS<sup>5)</sup>, the genus *Kriegeria* proposed by RABENHORST was informal and interim, and the description given by RABENHORST was made for

the species but not for the genus. GREMMEN<sup>8)</sup> followed in DENNIS' direction and reported three species of *Chloroscypha* from the Netherlands. It seems likely that *Kriegeria* took its qualification as the genus when SEAVER revived it.

Another question concerning the similarity between the species of the genus Chloroscypha and Ombrophila kriegeriana RABENH. was derived from the view of previous literature. SEAVER<sup>24</sup> thought that the species of Chloroscypha belonged to the same genus as Ombrophila kriegeriana RABENH. through the comparison with their description. It was stated by WINTER<sup>32)</sup> that morphologic characters of matured apothecia of the type specimen of Ombrophila kriegeriana RABENH., Fungi europaei No. 2315, quite agreed with those of Peziza elatina ALBERTINI et SCHWEINITZ, though they were almost immature. REHM<sup>17)18)</sup> reached the same conclusion as WINTER after examining RABENHORTS'S exsiccatus, Fungi europaei No. 2315, and treated it as a synonym of his new combination, Rutstroemia elatina (ALB. et Schw.) REHM. WHITE<sup>31)</sup>, who published a monograph of the genus Rutstroemia, also treated Ombrophila kriegeriana RABENH. as a synonym of R. elatina (ALB. et SCHW.) REHM. He also examined RABENHORST'S exsiccatus, Fungi europaei No. 2315. The genus Rutstroemia belongs to the family Sclerotiniaceae of the order Helotiales by the presence of its basal stroma formed in the substratum<sup>7)14)18)25)81).</sup> On the other hand, in the genus Chloroscypha the basal stroma was absent and it was placed in the family Helotiaceae of the order Helotiales<sup>7)14)25)</sup>. Therefore, there is considerable doubt whether or not the species of the genus Chloroscypha established by SEAVER and Ombrophila kriegeriana Apothecia of Ombrophila kriegeriana RABENH. as well as RABENH, belong to the same genus. Peziza elatina ALB. et SCHW. occur on twigs of Abies, whereas those of Chloroscypha occur on needles of many kinds of conifers except Abies.

From the points of view mentioned above, the author agrees with DENNIS'S opinion and accepts *Chloroscypha* SEAVER for the generic name of the needle blight fungi under consideration.

#### Identification of the species of genus Chloroscypha in Japan

As mentioned previously, SAWADA described three fungi which now would be applied to the genus Chloroscypha, as Mollisia as follows: Mollisia cryptomeriae SAWADA, M. chamaecyparidis SAWADA and M. thujopsidis SAWADA. Many fresh materials of Chloroscyphae were collected from various localities thoughout Japan, in addition to the specimens deposited in the herbarium. They were examined in detail and divided into three species in their morphologic characters, each of which agreed with three Mollisia descrided by SAWADA, respectively. It was revealed from the examination that these three fungi have respectively a wide host range and overlap each other in size of ascus and ascospore. Then, to distinguish them clearly from each other, some other features are to be utilized in combination with their size of ascus and ascospore. The important features utilized here are as follows: thickness of ascus tip, Melzer reagent\* reaction of apical pore of ascus, arrangement of ascospore in ascus, shape of ascospore, and length/width ratio of ascospore<sup>12)</sup>. Differences in these features among them are summarized in Table 1. Distribution of averaged size of ascus and ascospore from each material and of frequency in length, width, and length/width ratio of ascus and ascospore are given in Figures 1 to 6, respectively. Judged from these tables and figures, three species of the genus Chloroscypha

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<sup>\*</sup>KOH : 1.5g, I : 0.5g, Distilled water : 20cc, CCl<sub>3</sub> CHO • H<sub>2</sub>O : 22g

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Species Host			Chloroscypha seaveri (Reнм) Seaver	C. chamaecyparidis (Sawada) Kobayashi	C. thujopsidis (SAWADA) KOBAYASHI Thujopsis dolabrata SIEB. et Zucc. T. dolabrata var. hondai MAKINO Thuja standishii CARR. Cryptomeria japonica D. Don Chamaecyparis obtusa SIEB. et Zucc. C. pisifera ENDL.		
			Cryptomeria japonica D. Don Chamaecyparis obtusa SIEB. et Zucc.	Chamaecyparis obtusa Sieb. et Zucc. C. pisifera Endl. C. lawsoniana Parl.			
Ascus	Size	Range	105~160×17~28.5	105~155×14.5~23.5	80~135×15.5~21 μ		
		Average	133×22	129×18	102×18 μ		
	Length/width		6.1	7.1	5.6		
	Jode reaction of apical pore		-	+	+		
	Thickness of tip wall		5~15	2.5	2.5 μ		
Ascospore	Size	Range	18~34×7.5~11.5	13~23.5×9~13	15.5~23.5×6.5~9 $\mu$		
		Average	25×9	17×11	20×8 µ		
	Length/width		2.7	1.5	2.6		
	Arrangement		2	1	2		
	Shape		fusoid to boat-shaped	elliptic to globular	fusoid to boat-shaped		

# Table 1. Important characteristics to differentiate three species of *Chloroscypha* causing needle blight in Japan.

found in Japan would easily be differentiated from each other.

On the other hand, seven species of the genus *Chloroscypha* and *Kriegeria* have been known in North America and Europe. In order to compare these Japanese and foreign species which would belong to the genus *Chloroscypha*, morphologic characteristics and their host range are presented in Table 2.

As shown in Table 2, Mollisia cryptomeriae SAWADA<sup>10)12)20)</sup> quite agrees with not only Chloroscypha cryptomeriae TERRIER<sup>8)27)</sup> but also C. seaveri (REHM) SEAVER<sup>6)8)22)25)</sup>. TERRIER described it on the material collected on Cryptomeria japonica D. Don in Switzerland. He observed the thick wall of its ascus tip and negative jode reaction in apical pore of its ascus by means of the MELZER reagent. At the time when he identified the Chloroscypha on Cryptomeria, C. seaveri (REHM) SEAVER was only known on Thuja, and jode reaction of its ascus was not recorded. From these, TERRIER proposed his Chloroscypha as a new species and named it as C. cryptomeriae TERRIER. Recently, GREMMEN<sup>8)</sup> reported three species of the genus Chloroscypha, namely C. seaveri (REHM) SEAV. (on Thuja), C. sabinae (FUCK.) DENNIS (on Juniperus), and C. cryptomeriae TERRIER (on Cryptomeria), from the Netherlands. No details on their important characters being available to distinguish them, such as thickness of ascus tip, MELZER reagent reaction, etc., were given in this report. He regards these three species as separative for the time being, although their independency was doubted due to the great affinities between their morphologic characters, especially in size of ascus and ascospore. In the report, Mollisia cryptomeriae SAWADA

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		Worker	Ascus			Ascospore	
Species	Host		Size	Jode re- action	Thickness of tip wall	Size	Arrange- ment
Chloroscypha seaveri	Thuja plicata D. Don <sup>8)22)</sup>	Seaver <sup>22)25)</sup>	100~135×25~30			25~28×8~9	2
(Rehm) Seaver	T. occidentalis L. 19)25)	Dennis <sup>6)</sup>	80~90×15~20			24~30×8~12	2
	Chamaecyparis lawsonianaPARL <sup>5)~7)</sup>	Gremmen <sup>8)</sup>	108~135×32~35			27~29×10.5~12	
Mollisia cryptomeriae	Cryptomeria japonica D. Don <sup>10)12)20)</sup>	SAWADA <sup>20)</sup>	104~162×27~39			24~33×9~11	2
Sawada	Chamaecyparis obtusa S. et Z. <sup>12)</sup>	Іто <i>et al</i> <sup>10)</sup>	120~141×19~27			21~32×7~14	2
		Kobayashi <sup>12)</sup>	105~160×18~29		+	18~34×7.5~11.5	2
C. cryptomeriae	Cryptomeria japonica D. Don <sup>8)27)</sup>	TERRIER <sup>27)</sup>	115~140×19~24	-	+	20~31×6~12	2
Terrier		Gremmen <sup>8)</sup>	130~180×25~29			27~31×10.5~12	
C. cedrina (Cooke) Seaver	Juniperus virginiana L. <sup>23)</sup>	SEAVER <sup>23)25)</sup>	140~160×12~14			20×10	1
M. chamaecyparidis	Chamaecyparis obtusa S. et Z. <sup>12)</sup> C. pisifera Endl. <sup>21)</sup> C. lawsoniana PARL <sup>12)</sup>	SAWADA <sup>21)</sup>	83~114×16~18			16~20×9~10	1
Sawada		Kobayashi <sup>12)</sup>	105~155×15~23	+	-	13~23.5×9~13	1
M. thujopsidis	Thuja standishii CARR. <sup>21)</sup> Thujopsis dolabrata S. et Z. <sup>12)</sup> T. dolabrata var. hondai MAKINO <sup>21)</sup>	Sawada <sup>21)</sup>	94~145×11~22			14~19×6~10	2
Sawada	Cryptomeria japonica D. Don <sup>12)</sup> Chamaecyparis obtusa S. et Z. <sup>12)</sup> C. pisifera Endl. <sup>12)</sup>	Kobayashi <sup>12)</sup>	80~135×16~21	+	_	15.5~23.5×6.5~9	2
Kriegeria enterochloma (PECK) SEAVER	Thuja occidentalis L. <sup>25)</sup> T. orientalis L. <sup>25)</sup>	Seaver <sup>25</sup>	100~130×12~14			20~28×7~8	2
	Juniperus sabina L. <sup>6)7)</sup>	Dennis <sup>6)7)</sup>	90~150×14~20	-		15~21×6~8	2
C. sabinae (Fuck.) Dennis	J. nana Wild <sup>6)7)</sup> J. virginiana L. <sup>8)</sup> J. communis L. <sup>6)7)29)</sup>	Seaver <sup>22)25)</sup>	130×20			18~20×9~10	2
2 DINITS		Gremmen <sup>8)</sup>	145~150×18~21			21~23×9~10	
C. chloromella (Phill. et Hark.) Seaver	Sequoia sempervirens Endl. 22)25)	Seaver <sup>22)25)</sup>				20~25×6~7	
K. alutipes (Phill.) SEAVER	Libocedrus decurens Torrey <sup>25)</sup>	Seaver <sup>25)</sup>	90×10~12			18~20×5~8	

Table 2. Morphological characteristics of the fungi belonging to the genus Chloroscypha.

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Fig.1 Distribution of average size of ascus in each material of three *Chloroscypha* causing needle blight in Japan.

was considered as the same species as Chloroscypha cryptomeriae TERRIER, having in mind the description of the former fungus given by ITO et al<sup>10</sup>. On the other hand, DENNIS<sup>6</sup>) tested MELZER reagent reaction of Chloroscypha seaveri (REHM) SEAVER collected on Cupressus lawsoniana MURR. (=Chamaecyparis lawsoniana RARL.) in Britain and obtained negative results. In the case of Mollisia cryptomeriae SAWADA, the apical pore of its ascus reacted negative to the MELZER reagent and its ascus tip has thick wall<sup>12</sup>) as those of Chloroscypha cryptomeriae TERRIER. It was collected on not only Cryptomeria japonica D. DON but also Chamaecyparis obtusa SIEB. et Zucc.<sup>12</sup>). From these facts, it is quite clear that among the three fungi discussed here no difference is recognized in their morphology and host range. Then, Mollisia cryptomeriae SAWADA and Chloroscypha cryptomeriae TERRIER are treated as the synonyms of C. seaveri (REHM) SEAVER.

Mollisia chamaecyparidis SAWADA<sup>12)21)</sup> and M. thujopsidis SAWADA<sup>12)21)</sup> seem to be similar in their morphologic characters to Chloroscypha cedrina (COOKE) SEAVER<sup>23)</sup> and Kriegeria enterochloma (PECK) SEAVER<sup>25)</sup>, respectively. Mollisia chamaecyparidis SAWADA was collected on Chamaecyparis spp. <sup>10)21)</sup>, but Chloroscypha cedrina (CKE.) SEAVER on Juniperus virginiana L.<sup>23)</sup> In the case of Mollisia thujopsidis SAWADA and Kriegeria enterochloma (PECK) SEAVER, Thuja is the common host plant<sup>12)21)25)</sup>. In the case of Mollisia chamaecyparidis SAWADA and M. thujopsidis SAWADA, the apical pore of their ascus reacted positive and blue-stained with the Melzer reagent<sup>12)</sup>, whereas SEAVER did not confirm jode reaction in the ascus of Chloroscypha cedrina (CKE.) SEAVER and Kriegeria enterochloma (PECK) SEAVER. Although the difference of the host range is not greatly important



Fig. 2 Frequency of length and width of ascus in three *Chloroscypha* causing needle blight in Japan (Total numbers of measured ascus).



Fig. 3 Frequency of length/width ratio of ascus in three *Chloroscypha* causing needle blight in Japan (Total numbers of measured ascus).

in separating the species in the genus *Chloroscypha*, MELZER reagent reaction is one of the important features to distinguish them. Then, the two Japanese species described by SAWADA are regard as the other species from SEAVER's two species, respectively, and their generic name is alternated from *Mollisia* to *Chloroscypha*.



Fig. 4 Distribution of average size of ascospore in each material of three *Chloroscypha* causing needle blight in Japan.

Chloroscypha Seaver, Mycol. 23: 248, 1931; Dennis, Kew Bull. 3: 410, 1954.

Syn. Kriegeria RABENHORST SENSU SEAVER, Mycol. 35: 492, 1943.

1. Chloroscgpha seaveri (REHM)SEAVER----(Plates 1:A, B, 2:A-D; Fig. 7)----Mycol. 23:249, 1931; Dennis, Kew Bull. 3:410, 1954; Dennis, Commonw. Mycol. Inst. Mycol. Pap. 62:123, 1956: Gremmen, Nova Hedw. 6:21~22, 1963.

Syn. *Mollisia cryptomeriae* SAWADA, Bull. Gov. For. Exp. Sta. (Tokyo) 45:34, 1950; Ito *et al.*, Bull. Gov. For. Exp. Sta. (Tokyo) 52:126~131, 1952; KOBAYASHI, Jour. Jap. For. Soc. 46:338~343, 1964.

Chloroscypha cryptomeriae TERRIER, Ber. Schw. Bot Ges. 62:422, 1952; GREMMEN, Nova Hedw. 6:22~23, 1963.

Apothecium on needle, single or aggregate, non- or short-stipitate, black and turbinate under dry condition, cup- to disk-shaped and yellowish green to olive under moist condition,  $0.5 \sim 0.7$ mm in diameter, 0.3mm in height. Ectal excipulum  $70 \sim 80\mu$  thick, prosenchymatous, divided into two layers, inner layer composed of dense slender hyphae arranged parallel upwards, and outer layer composed of a loose one and filled with gelatinous substance. Epithecium  $13 \sim 18\mu$  thick, olivish, constituted from small angular cells and coloured tip of paraphyses. Hymenium hyaline, formed with asci and paraphyses in a row. Hypothecium and medullary excipulum a little. Ascus hyaline, cylindric to clavate,  $105 \sim 160 \times 18 \sim 28.5\mu$ , usually  $120 \sim 140 \times 20 \sim 24\mu$ , 8-spored, wall  $1 \sim 2.5\mu$  thick at the side,  $5 \sim 15\mu$  thick at the tip, apical pore not blued with the MELZER reagent. Ascospore biseriate, unicellular, hyaline to pale yellowish green, fusoid to boat-shaped, sometimes

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Fig.5 Frequency of length and width of ascospore in three *Chloroscypha* causing needle blight in Japan (Total numbers of measured ascospore).



Fig.6 Frequency of length/width ratio of ascospore in three *Chloroscypha* causing needle blight in Japan (Total numbers of measured ascospore).

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Fig. 7 Chloroscypha seaveri (REHM) SEAVER

A: Schematic figure of apothecium (a: epithecium, b: hymenium, c: hypothecium and medullary excipulum, d: ectal excipulum, e: stalk) B: A part of apothecium (a: outer layer of ectal excipulum, b: inner layer of ectal excipulum, c: hymenium, d: epithecium) C: Asci and ascospores D: Paraphyses E: Ascospores F: Germinating ascospores

elliptic,  $18 \sim 34 \times 7.5 \sim 11.5\mu$ , usually  $23.5 \sim 26 \times 8 \sim 10.5\mu$ . Paraphysis hyaline, filiform, usually branched, swollen and pale olivish at the tip,  $125 \sim 165\mu$  in length.

Host: on blighted or self-prunned dead needles of *Thuja plicata* D. Don<sup>22)</sup>, *T. occidentalis* L. (Nioi-hiba)<sup>19)24)</sup>, *Chamaecyparis lawsoniana* PARL. (Bei-hi)<sup>5)-7)12)</sup>, *C. obtusa* SIEB. et Zucc. (Hinoki)<sup>12)</sup>, and *Cryptomeria japonica* D. -Don (Sugi)<sup>10)12)20)</sup>,

**Distribution :** North America (United States<sup>22)</sup> and Canada<sup>19)24)</sup>), Europe (England<sup>5)~7)</sup>, Switzerland<sup>27)</sup> and Netherlands<sup>8)</sup>), and Asia (Japan<sup>10)12)20)</sup>).

Material: Cryptomeria japonica D. Don-Yahaba, Iwate Pref., V-28, 1949, by K. Sawada

(Paratype); Toribuchi, Gunma Pref., VII-7, 1951, by S. Колтали; Kaminoyama, Yamagata Pref., XI-2, 1954, by K. Ito; Shidara, Aichi Pref., V-17, 1963; V-26, 1964, by T. Коватаяні; Nippara, Tokyo, XI-9, 1963, by T. Коватаяні; Kitafusa, Okayama, Pref., XI-21, 1963, by S. Kontani; Kishuku, Nagasaki Pref., III-6, 1964, by Y. Токизнісє; Okutama, Tokyo, IV-, 1964; Ikawa, Shizuoka Pref., V-6, 1964, by S. Mizumoto; Nakano, Yamanashi Pref., V-8, 1964, by Y. Zinno; Hikawa, Tokyo, V-13, 1964, by O. Сніва; Kurokawa, Gifu Pref., V-, 1964, by Y. Morimoto; Kanuma, Tochigi Pref., VI-6, 1964, by O. Сніва. *Chamaecyparis obtusa* Sieb. et Zucc. — Momoyama, Kyoto, IV-15, 1964, by S. Kontani; Asakawa, Tokyo, IV-23, 1964, by T. Kobayashi; Shidara, Aichi Pref., V-26, 1964, by T. Kobayashi.

Besides the above-listed localities, the fungus has been recorded hitherto from several localities in Japan as follows: Aomori<sup>20)</sup>, Iwate<sup>10)20)</sup>, Akita<sup>10)20)</sup>, Yamagata<sup>10)20)</sup>, Fukushima<sup>20)</sup>, Miyagi<sup>10)</sup>, Saitama<sup>9)15)</sup>, Gunma<sup>9)</sup>, Okayama<sup>18)26)</sup>, Ehime<sup>10)</sup>, and Kochi<sup>30)</sup> prefectures.

Note: Since the first record of this fungus in 1950 in Japan, outbreaks of the needle blight of *Cryptomeria japonica* D. Don caused by it were recorded in the forest stands of Tochigi, Saitama and Gunma prefectures in 1951<sup>9)15)</sup>, and in the Kanto and Chubu districts in 1964<sup>12)</sup>.

Symptoms first appear in early spring on the 1-year-old needles of under part of the crown, and the disease immediately spreads outerwards of the crown until July. Affected needles are soon killed and later twigs are also killed. Then, the heavily affected trees become reddish brown except the outermost part of the crown, and from a distance give an appearance of having been scorched by fire or injured by late frost. Branches survive and give forth adventitious buds. In August, affected trees recover their green appearance as a result of the growth of the new shoots. Apothecia are formed on the blighted needles and many matured ones are recognized from April to July. Almost all of them fall off in August and only black dots are found on these needles.

The present *Chloroscypha* may not cause the needle blight on *Chamaecyparis obtusa* SIEB. et Zucc. It was recognized only on the self-prunned dead needles of *Chamaecyparis obtusa* SIEB. et Zucc., even though they were grown adjacent to the heavily diseased stands of *Cryptomeria japonica* D. Dow.

Several attempts to isolate this fungus were made and its ascospore germinated only once on *Cryptomeria* needle decoction agar. Its colony developed on the transferred potato-sucrose agar slant. Growth of the colony on this medium was very slow as noted by KONTANI and MINEO<sup>13)</sup>, and GREMMEN<sup>8)</sup>. Though they obtained the culture of this fungus, their notes on the isolation of the fungus indicated that there were certain difficulties in obtaining it. Reason for the uncertainty in germination of ascospore of the present fungus has not been explained.

2. Chloroscypha chamaecyparidis (SAWADA) T. KOBAYASHI, comb. nov. ----(Plates 3, Fig. 8)

Syn. Mollisia chamaecyparidis SAWADA, Bull. Gov. For. Exp. Sta. (Tokyo) 46:126, 1950; Kobayashi, Jour. Jap. For. Soc. 46:339~342, 1964.

Apothecium on needle, single or aggregate, black and turbinate under dry condition, cup- to disk-shaped and yellowish green to pale olive under moist condition,  $0.5\sim1$ mm in diameter,  $0.1\sim0.2$  mm in height, non- or short-stipitate. Ectal excipulum  $40\sim80\mu$  thick, prosenchymatous, divided into two layers, inner layer composed of dense slender hyphae arranged parallel upwards, outer layer composed of a loose one and filled with gelatinous substance. Epithecium  $15\sim40\mu$  thick, pale olive to yellowish green, constituted from small angular cells and coloured tip of paraphyses. Hymenium hyaline, formed with asci and paraphyses in a row. Stalk, if recognized,  $90\sim220\mu$ 

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Fig. 8 Chloroscypha chamaecyparidis (SAWADA) KOBAYASHI A: Schematic figure of apothecium B: A part of apothecium C: Asci and ascospores, apical pores blued with MELZER reagent D: Paraphyses E: Ascospores F: Germinating ascospores

in diameter,  $110 \sim 220\mu$  in length. Ascus hyaline, cylindric to clavate,  $105 \sim 155 \times 14.5 \sim 23.5\mu$ , usually  $120 \sim 140 \times 16 \sim 21\mu$ , 8-spored, wall  $1 \sim 2.5\mu$  in thickness, apical pore blued with MELZER reagent. Ascospore hyaline to pale yellowish green, uniseriate, rarely irregularly biseriate, unicellular, elliptic to globular, rounded at the ends,  $13 \sim 23.5 \times 9 \sim 13\mu$ , commonly  $14.5 \sim 18 \times 10.5 \sim 11.5\mu$ . Paraphysis hyaline, filiform, usually branched, swollen and olivish at the tip,  $150 \sim 170\mu$  in length.

Host : on blighted or self-prunned dead needles of *Chamaecyparis pisifira* ENDL. (Sawara)<sup>12)21)</sup>, *C. obtusa* SIEB. et Zucc. (Hinoki)<sup>12)</sup>, and *C. lawsoniana* PARL. (Bei-hi)<sup>12)</sup>.

Distribution : Asia (Japan<sup>12)21)</sup>).

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Material : Chamaecyparis pisifera ENDL. — Nishi-nasuno, Tochigi Pref., VII-13, 1964, by T. KOBAYASHI. C. obtusa SIEB. et Zucc. — Ueno, Chiba Pref., VI-14, 1954, by T. KOBAYASHI ; Otaki, Shiga Pref., VI-, 1954 ; Hachioji, Tokyo, VI-20, 1955, by Y. ZINNO ; Asakawa, Tokyo, VI-29, 1955, by Y. ZINNO ; Ikawa, Shizuoka Pref., VII-15, 1960 ; Uratakao, Tokyo, IV-23, 1964, by T. KOBAYASHI ; Nishi-nasuno, Tochigi Pref., VII-13, 1964, by T. KOBAYASHI ; Mt. Keicho, Tochigi Pref., VII-14, 1964, by T. KOBAYASHI. C. lawsoniana PARL. — Kiyomi, Gifu Pref., VII-16, 1962, by Y. MORIMOTO.

SAWADA first described this species on *Chamaecypacis pisifera* ENDL. collected from Iwate and Akita prefectures.

Note: This fungus was collected only on *Chamaecyparis* spp. An outbreak of the needle blight caused by the present *Chloroscypha* was recorded in 1954 on *Chamaecyparis obtusa* SIEB. et Zucc. in nurseries and forest stands. Two-year-old seedlings in two nurseries of Chiba prefecture were severely attacked and one hundred thousand of the seedlings became useless during the year. Symptoms on them appeared in the early spring. The disease spreads immediately and affected needles fall to the ground. Twigs gradually die by the loss of their needles and heavily affected seedlings finally die. In the case of the plantations, extremely closed forests are often affected severely. Damaged trees did not die but they lost a half or more branches of the crown by the needle blight. In this year, extreme drought was recorded in spring. There were no rainfalls for about a month after transplanting the seedlings. It is assumed from the foregoing that a weakened condition caused by the drought stimulated the development of the needle blight.

In the case of *Chamaecyparis pisifera* ENDL. the fungus was collected only on its dead needles in hedges.

This fungus was also found in a forest of *Chamaecyparis lawsoniana* PARL. planted experimentally in Gifu prefecture. It causes severe needle blight on the host together with another needle blight fungus, whose systematic position has not yet been determined. Occurrence of the mixed infection was first discovered in 1960 and has been continuously observed every year since.

In several attempts to isolate the present *Chloroscypha*, its ascospore germinated only once on *Cryptomeria* decoction agar. Germinating ascospore swelled, became greenish brown and bore its germ-tube everywhere. However, germinated ascospore which was transplanted to potato-sucrose agar failed to develop its colony.

3. Chloroscypha thujopsidis(SAWADA)T. KOBAYASHI, comb. nov. — (Plates, 2 : E-G, Fig. 9)

Syn. Mollisia thujopsidis Sawada, Bull. Gov. For. Exp. Sta. (Tokyo) 46:141, 1950; Kobayashi, Jour. Jap. For. Soc. 46:339~343, 1964.

Apothecium on needle, hypophyllous on *Thujopsis*, amphigenous on the other hosts, single or aggregate, black and turbinate under dry condition, cup- to disk-shaped and yellowish green to olivish under moist condition,  $0.4 \sim 0.6$ mm in diameter, non- or short-stipitate. Ectal excipulum olivish, prosenchymatous, divided into two layers, inner layer composed of dense slender hyphae arranged parallel upwards, outer layer composed of loose hyphae and filled with gelatinous substance. Epithecium olivish, constituted from small angular cells and coloured tip of paraphyses. Hymenium hyaline, formed with asci and paraphyses in a row. Hypothecium and medullary excipulum a little. Ascus hyaline, cylindric to clavate,  $80 \sim 135 \times$  $15.5 \sim 21\mu$ , usually  $90 \sim 120 \times 15.5 \sim 21\mu$ , 8-spored, apical pore blued with the MELZER reagent, wall

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Fig. 9 Chloroscypha thujopsidis (SAWADA) KOBAYASHI A: Schematic figure of apothecium B: A part of apothecium C: Asci and ascospores, apical pores blued with Melzer reagent D: Paraphyses E: Ascospores

 $1\sim2.5\mu$  in thickness. Ascospore biseriate, unicellular, hyaline to pale yellowish green, fusoid to boat-shaped, sometimes elliptic,  $15.5\sim23.5\times6.5\sim9\mu$ , usually  $18\sim21\times7.5\sim8\mu$ . Paraphysis hyaline, filiform, usually branched, swollen and olivish at the tip,  $100\sim150\mu$  in length.

Host: on blighted or self-prunned dead needles of *Thujopsis dolabrata* SIEB. et Zucc. (Hiba)<sup>12)</sup>, *T. dolabrata* var. *hondai* MAKINO (Hinoki-asunaro)<sup>21)</sup>, *Thuja standishii* CARR. (Nezuko)<sup>21)</sup>, *Chamaecyparis obtusa* SIEB. et Zucc. (Hinoki)<sup>12)</sup>, *C. pisifera* ENDL. (Sawara)<sup>12)</sup>, and *Cryptomeria japonica* D. Don (Sugi)<sup>12)</sup>.

Distribution : Asia (Japan<sup>12)21)</sup>).

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Material: Thujopsis dolabrata SIEB. et Zucc. — Shizukuishi, Iwate Pref., V-11, 1964, by K. SATO; Morioka, Iwate Pref., VI-23, 1964, by K. SATO. Cryptomeria japonica D. Don—Kitagawa, Miyazaki Pref., V-11, 1964, by Y. YOSHINAGA. Chamaecyparis obtusa SIEB. et Zucc. — Fushimi, Kyoto, IV-15, 1964, by S. KONTANI. C. pisifera ENDL. — Nishi-nasuno, Tochigi Pref., VII-13, 1964, by T. KOBAYASHI.

SAWADA described this species on *Thujopsis dolabrata* var. *hondai* MAKINO collected in Aomori and Akita prefectures, and on *Thuja standishii* CARR. collected in Miyagi prefecture.

Note: No serious outbreak of the needle blight caused by the present fungus has been recorded up to the present. On *Thujopsis dolabrata* SIEB. et Zucc. it causes needle blight, but does not cause heavy damage. The fungus was also collected on *Cryptomeria japonica* D. Don in Kyushu. Symptoms caused by the present species on them are quite similar to those caused by *Chloroscypha seaveri* (REHM) SEAVER. Two species of *Chloroscypha* on *Cryptomeria* are to be differentiated only by microscopic examination of each other.

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The fungus may inhabit saprophytically *Chamaecyparis obtusa* SIEB. et Zucc. and *C. pisifera* ENDL.

Isolation of this species was unsuccessful. Ascospore could not be germinated on any tested agar media.

#### Summary

1. Systematic position of three needle blight fungi on several kinds of Japanese conifers which had been previously regarded as the species of the genus *Mollisia* by SAWADA, was restudied, and *Chloroscypha* SEAVER was accepted as the generic name for them.

2. Several characteristics which are recognized under microscope, such as size of ascus and ascospore, thickness of ascus wall at the tip, MELZER reagent reaction of apical pore of ascus, arrangement of ascospore in ascus, and shape and length/width ratio of ascospore, are available to identify the species of *Chloroscypha* causing the needle blight in Japan.

3. Of three fungi restudied here, *Mollisia cryptomeriae* SAWADA was treated as a synonym of *Chloroscypha seaveri* (REHM) SEAVER. *Chloroscypha cryptomeriae* TERRIER described by TERRIER from Switzerland was also treated as a synonym of it. The other two fungi remained as independent species, respectively, but they were recombined under the genus *Chloroscypha* from *Mollisia* respectively as *C. chamaecyparidis* (SAWADA) KOBAYASHI and *C. thujopsidis* (SAWADA) KOBAYASHI.

4. These three Chloroscyphae have a wide host-range and, in general, inhabit harmlessly on the self-prunned dead needles of several kinds of conifers. However, they sometimes cause severe needle blight on particular host plants. Chloroscypha seaveri (REHM) SEAVER causes severe needle blight on Cryptomeria japonica D. Don, but not on Chamaecyparis obtusa SIEB. et Zucc. In the cases of Chamaecyparis obtusa SIEB. et Zucc. and C. lawsoniana PARL. the needle blight is caused by Chloroscypha chamaecyparidis (SAWADA) KOBAYASHI, though the other two species inhabit saprophytically on them. Chloroscypha thujopsidis (SAWADA) KOBAYASHI causes commonly the needle blight of Thujopsis dolabrata SIEB. et Zucc. It is found saprophytically on Chamaecyparis obtusa SIEB. et Zucc. and C. pisifera ENDL. It is observed in rare cases that Chloroscypha thujopsidis (SAWADA) KOBAYASHI causes the needle blight of Cryptomeria japonica D. Don which is quite similar to that caused by C. seaveri (REHM) SEAVER on the same host.

5. Worth noting in the fact that there was great uncertainty in the germination of their ascospore. Almost all of the attempts to isolate these fungi failed, since their ascospores did not germinated on any kind of agar media tested. Germination of ascospore was obtained only twice. Reason for the capricious germination of ascospore in these fungi has not been explained.

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### **Explanation** of plates

## Plate 1

Chloroscypha seaveri (Rehm) Seaver

- A: A diseased forest of Cryptomeria japonica D. Don (Danto National Forest., Photo. V-26, 1964).
- B: Apothecia produced on the infected needle of Cryptomeria japonica D. Don

#### Plate 2

Chloroscypha seaveri (REHM) SEAVER

A : Infected needles of Cryptomeria japonica D. Dow showing apothecia as black dots. ×1

B : Do. Enlarged.  $\times 2$ 

C ; Apothecium.  $\times 130$ 

D : Ascus having thick wall at the tip.  $\times 500$ 

Chloroscypha thujopsidis (SAWADA) KOBAYASHI

- E : Infected needles of *Thujopsis dolabrata* SIEB. et Zucc. showing apothecia as black dots. ×1.5
- F : Infected needles of Cryptomeria japonica D. Don showing apothecia as black dots.  $\times 2$

G: Ascus having thin wall and blue-stained apical pore.  $\times 500$ 

## Plate 3

Chloroscypha chamaecyparidis (Sawada) Kobayashi

- A : Infected needles of *Chamaecyparis obtusa* SIEB. et Zucc. showing apothecia as black dots. ×2
- B : Infected needles of Chamaecyparis lawsoniana RARL. showing apothecia as black dots.  $\times 2.5$
- C: Apothecium produced on Chamaecyparis obtusa SIEB. et Zucc. ×130
- D: Apothecium produced on Chamaecyparis lawsoniana PARL. ×90
- E : Clushed apothecium showing many asci in circle.  $\times 150$
- F : Ascus having thin wall.  $\times 500$

針葉樹黒粒葉枯病菌の属ならびに種の 分類同定に関する再検討 小林享、夫<sup>∞</sup>

# (要旨)

現在わが国には針葉樹の黒粒葉枯病菌の病原菌として3種の菌が知られている。すなわち,スギ黒粒葉枯 病菌(Mollisia cryptomeriae SAWADA)<sup>20)</sup>, ヒノキ(サワラ)黒粒葉枯病菌(M. chamaecyparidis 'SAWADA)<sup>21)</sup>, およびヒノキアスナロ(ヒバ)黒粒葉枯病菌(M. thujopsidis 'SAWADA)<sup>21)</sup> である。このうちスギ黒粒葉枯 病菌によると思われるスギの病害は、すでに1920年代に北島<sup>11)</sup> によって各地から報告され、また別に富 樫ら<sup>28)29)</sup> によって岩手県下から報告されている。これらの報告では病原菌の種の同定はおこなわれず、 1950年沢田によってはじめて命名記載された。しかし、近年になって欧米の針葉樹に日本産の黒粒葉枯病 菌によくにた葉枯性病菌が数種あり、それらには Mollisia とは異なる属名が用いられていることがわか った。そこでこれら日本産ならびに欧米産の葉枯性病菌の異同と所属について検討を加えることとし、研 究室保存標本および新たに各地から採集された標本の調査をおこない、日本産の黒粒葉枯病菌の所属と類 別をあきらかにした。

国内各地から採集されたスギ15, ヒノキ12, サワラ2, ベイヒ1, ヒバ2の計32標本の調査の結果,日本産の黒粒葉枯病菌は,沢田が記載したように3種に類別されるが<sup>12)</sup> (Table 1, Figs. 1~6), それらは 子のう盤の構造,子のう胞子の形状などから,あきらかに Mollisia 属ではなく,北米では Kriegeria, 欧州では Chloroscypha として用いられている属に所属する。1931年にこの群の菌類をまとめて Chloroscypha 属を創設した SEAVER<sup>22)</sup> は,のち文献により Chloroscypha 属の菌は1887年に記載された Ombrophila? kriegeriana RABENHORST<sup>16)</sup> と同じ特徴を有するとし,この種の記載のあとがきを正式な記述と認 め Kriegeria RABENHORST を属名として採用し, Chloroscypha SEAVER をその異名とした<sup>24)</sup>。「Dictionary of fungi |<sup>2)</sup> は SEAVER にしたがって Kriegeria 属を採用している。

一方、DENNIS<sup>5)</sup>は RABENHORST のあとがきはかりの意見であって正式な属の記載ではないとして SEAVER の属名変更を認めず、*Chloroscypha* SEAVER を正名として用いた。GREMMEN<sup>8)</sup>は DENNIS にしたがい *Chloroscypha* 属を用いている。筆者も DENNIS と同じく、RABENHORST の記載は種の記載であり、そのあ とがきは属の記載とは認め難いと考える。さらに *Ombrophila kriegeriana* RABENH.の標本を直接しらべ た WINTER<sup>82)</sup>、REHM<sup>17)18)</sup>、WHITE<sup>81)</sup>らはいずれもこの菌を *Rutstroemia elatina* (ALB. et Schw.) REHM と同一菌とし、その異名とした。*Rutstroemia* 属は子のう盤が寄主組織内の子座から生じ Sclerotiniaceae に所属する<sup>7)14)18)25)81)が、*Chloroscypha* 属にはこのような子座 はなく Helotiaceae に所属する<sup>7)14)25)。 したがって、SEAVER が Kriegeria 属のタイプ種とした Ombrophila kriegeriana RABENH. を、*Chloroscypha*</sup></sup>

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属の菌と同一属とするには大きな疑問があり、属の資格についての疑義とともに Kriegeria 属を用いるのは不適当と考える。これらの点から、黒粒葉枯病菌の属名には Dennis と同じく Chloroscypha Seaver を採用する。

次に日本産および欧米産の黒粒葉枯病菌の異同を比較するため、それらの主要な形態的特徴と寄主を Table 2 にしめした。表にみるごとく、スギ黒粒葉枯病菌は1952年にスイスでスギ上に記載された Chloroscypha cryptomeriae TERRIER によく一致するとともに Chloroscypha 属のタイプ種 C. seaveri (REHM) SEAVER にもよく一致する。最近 GREMMEN<sup>8)</sup> はオランダから3種の Chloroscypha を報告したが、そのな かで伊藤ら<sup>11)</sup>の記載によって Mollisia cryptomeriae SAWADA を Chloroscypha cryptomeriae TERRIER と 同一菌と考えた。現在のうえにあげた3種の菌は、子のう頂孔が MELZER 試薬に青染しないこと、子のう 頂膜が著しく厚いこと、子のう胞子の形、大きさ、ならび方などの点でまったく一致し同一種と考えられ るので、 Mollisia cryptomeriae SAWADA および Chloroscypha cryptomeriae TERRIER をいずれも C. seaveri (REHM) SEAVER の異名として処理した。ヒノキ黒粒葉枯病菌およびヒバ黒粒葉枯病菌は、それぞれ Chloroscypha cedrina (Cooke) SEAVER および Kriegeria enterochloma (PECK) SEAVER に似るが、あとの2菌 については MELZER 試薬反応が不明であるので、沢田の記載した2種はそれぞれ独立種として残し、属名 変更にとどめた。以上によって日本産黒粒葉枯病菌の属、種名を整理して次にしめす。

Chloroscypha SEAVER, Mycol. 23: 248, 1931, DENNIS, Kew. Bull. 3: 410, 1954.

異名: Kriegeria RABENHORST SENSU SEAVER, Mycol. 35:492, 1943.

1. Chloroscypha seaveri (REHM) SEAVER— (Plate 1, 2, A~D, Fig. 7) — Mycol. 23: 249, 1931; DENNIS, Kew. Bull. 3:410, 1954; DENNIS, Commonw. Mycol. Inst. Mycol. Pap. 62: 123, 1956; GREMMEN, Nova Hedw. 6: 21~22, 1963.

異名: Mollisia cryptomeriae SAWADA,林試研報 45:34, 1950;伊藤ら,林試研報 52:126~131, 1952; 小林,日林誌 46:339~343, 1964.

Chloroscypha cryptomeriae Terrier, Ber. Schw. Bot. Ges. 62:421, 1952; GREMMEN, Nova Hedw. 6:22~23, 1963.

寄主: Thuja plicata D. Don<sup>22)</sup>, ニオイヒバ<sup>19)25)</sup>, ベイヒ<sup>5)~7)</sup>, ヒノキ<sup>12)</sup> およびスギ<sup>10)12)20)</sup>の針葉に 生ずる。

分布:北米 (アメリカ<sup>22)</sup>, カナダ<sup>19)25)</sup>), 欧州 (イギリス<sup>5)~7)</sup>, スイス<sup>27)</sup>, オランダ<sup>8)</sup>) およびアジア (日本<sup>10)12)20)</sup>。

和名:スギ黒粒葉枯病菌<sup>9)</sup>

Chloroscypha chamaecyparidis (SAWADA) T. KOBAYASHI, comb. nov. — (Plate 3, Fig. 8)

異名: *Mollisia chamaecyparidis* SAWADA,林試研報46:126,1950;小林,日林誌46:339~343,1964. 寄主:サワラ<sup>12)21)</sup>,ヒノキ<sup>1)12)</sup> およびベイヒ<sup>12)</sup> の針葉に生ずる。

分布:アジア (日本12)21))。

和名;ヒノキ黒粒葉枯病菌。沢田<sup>21)</sup>はサワラ黒粒葉枯病菌と命名したが,一般にはヒノキ黒粒葉枯病菌<sup>1)</sup> として用いられている。本菌による黒粒葉枯病はヒノキにもっとも重要であるから,ヒノキ黒粒葉枯病菌

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を残した。

3. Chloroscypha thujopsidis (SAWADA) T. KOBAYASHI, comb. nov. — (Plate 2 : E~G, Fig.
9)

異名: *Mollisia thujopsidis* SAWADA,林武研報 46:141,1950;小林,日林誌 46:339~343,1946. 寄主:ヒ<sup>パ12)</sup>,ヒノキアスナロ<sup>21)</sup>,ネズコ<sup>21)</sup>,ヒノキ<sup>12)</sup>,サワラ<sup>12)</sup>およびスギ<sup>12)</sup>の針葉に生ずる。 分布:アジア(日本<sup>12)21</sup>)

和名:ヒバ黒粒葉枯病菌。沢田<sup>21)</sup>はヒノキアスナロ黒粒葉枯病菌と命名したが、ヒノキアスナロはヒ バの変種であること、寄主の重要性などから、本菌をヒバ黒粒葉枯病菌と改めたい。

これら3種の黒粒葉枯病菌は、寄主範囲がひろくたがいに重複するが、いままでのところスギ黒粒葉枯 病菌がスギに<sup>9)19)15)</sup>、ヒノキ黒粒葉枯病菌がヒノキ<sup>1)12)</sup>に大発生して問題になったほかは、特に病害とし て問題になった記録はなく、今回の調査においても、他の樹種上では下枝の枯死針葉上に腐生的に生じた ものがほとんどであった。ただヒバ黒粒葉枯病菌がスギ上にスギ黒粒葉枯病菌によるのとよく似た病状を しめした1例があり、あるいは今後他の樹種上でも問題になる可能性がないとはいえない。

黒粒葉枯病菌の子のう胞子は、かなり容易に放出されるにもかかわらず、寒天培地上で発芽させること がなかなかむずかしいもののようである。このことは紺谷・峰尾<sup>13)</sup> および GREMMEN<sup>8)</sup> の報告からもうか がえるが、筆者のおこなった多くの分離実験のうち、発芽したのは2回のみであった。 針葉樹黒粒葉枯病菌の属ならびに種の分類同定に関する再検討(小林) -Plate 1-







# 針葉樹黒粒葉枯病菌の属ならびに種の分類同定に関する再検討(小林) ―Plate 3―



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