

## Fungus Isolates from Japanese Black and Red Pine Seeds with Some Taxonomical Notes

By

Tsuneo WATANABE<sup>(1)</sup>, Seiji UEMATSU<sup>(2)</sup>  
and Yukio SATO<sup>(3)</sup>

**Summary :** Fungus floras in Japanese black and red pine seeds derived from six different locations in Japan were studied with special attention to frequencies of isolation in the respective samples and taxonomy of individual isolates. A total of 1,615 fungal isolates (149—491 isolates per sample) were obtained by assaying 2,000 seeds (300—500 seeds per sample). To obtain as many species as possible, seeds assayed were only washed and not surface sterilized before plating. These fungi were classified into nearly 53 species, 33 types and 3 groups belonging to 41 genera excluding unsporulated and unidentified isolates. Fourteen genera were frequently isolated from both or either seed of black and red pines. They were *Alternaria*, *Arthrinium*, *Aspergillus*, *Aureobasidium*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Penicillium*, *Pestalotia*, *Phialophora*, *Phoma*, *Trichoderma*, and *Tripodermum*. *Diplodia*, *Phomopsis* and unidentified  $X_1$  were also frequently isolated in particular samples. Some isolates including *Camposporium*, *Humicola* and *Pyrenochaeta* may possibly be new species. It was suggested that there must be present some seed-borne plant pathogens among our isolates because one isolate each of *Pestalotia* and *Phomopsis* showed pathogenicity on young pine seedlings by inoculation experiments.

### Introduction

Fungus floras associated with seeds of *Pinus* spp. have been studied in foreign countries mostly in relation to the study on seed-borne plant pathogens. For example, 15 genera of fungi were found associated with seeds of *Pinus caribaea* in Tanzania (HOCKING 1968). In an annotated list of seed-borne diseases compiled by RICHARDSON in 1979, 15 fungus genera or species were recorded on *Pinus* seeds.

However, no fungi inhabiting pine seeds have been studied in Japan although some 10 genera were listed as the seed-borne fungi on other forest seeds including Japanese cypress (SATO 1955, SATO & OHTA 1953, SATO & SHOJI 1960).

This study was conducted to obtain additional data on the fungus floras associated with various forest seeds in Japan. This is the first report concerned with the fungi on Japanese black and red pine seeds. In addition, pathogenicity of two isolates each of *Pestalotia* and *Phomopsis* on to pine seedlings was also discussed.

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(1) Forest Protection Division

(2) Chiba Horticultural Experiment Station

(3) Toyama Prefectural College of Technology

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### Materials and Methods

Six lots of Japanese black pine (*Pinus thunbergii* PARL.) and Japanese red pine (*P. densiflora* SIEB. et ZUCC.) seeds were used for this study. Among them, seeds of two lots (Nos. 13 and 24) of red pine that were collected at Higashi-Chikuma and Kami-Ina, Nagano Pref. and stored at 5°C were donated by Shunsuke OKUMURA, Nagano Prefectural Forest Experiment Station in September, 1984. The other 4 lots of black and red pine seeds, stored for 2–5 years at –5°C in the Experimental Forest Laboratory, Forestry and Forest Products Research Institute, were used for this study. These are seeds of black pine with the lot number 79–186 collected at Kumano, Mie Pref. and 81–026 at Ohkawa, Kagawa Pref., and of red pine with the lot number 81–038 at Hiba, Hiroshima Pref. and 83–073 at Seihaku, Tottori Pref.. Germination rates of all of these seeds ranged from 58–97% on water agar in plates (2 seeds/plate) after 15 day incubation at 25°C (Table 1).

Isolations were conducted by hyphal tipplings from the mycelial growth from seeds placed on 7 ml water agar in 9 cm petri dishes (2 seeds/plate) and incubated at 25°C for 2–4 days and 7° or 10°C for 7–10 days. Seeds were previously well washed for more than 30 min under running tap water and subsequently air dried by removing free water.

A total of 2,000 seeds of 6 lots, 300 seeds for each seed lot except a sample, no. 24 of Nagano in which 500 seeds were assayed were used for this study. Namely, the fungi were isolated at 25°C from 150 seeds (250 for the no. 24 of Nagano) of each seed lot and at 7°C for the rest of 150 (250 for the no. 24) in at least four separate experiments. These seeds were contaminated with fungi at the rates of 38.7 to 100% at 25°C (Table 1).

Pathogenicity of two isolates each of *Pestalotia* and *Phomopsis* were tested. The isolates used were 83–2 and 83–3 for *Pestalotia*, and 84–517 and 84–520 for *Phomopsis*. Among these isolates, 83–3 was isolated from black pine seed, and the rest from red pine seed. Inocula were prepared by brushing off spores from colony surface of 1-month old slant cultures after addition of sterilized distilled water (10 ml/tube), and filtering through double layers of gauze. Inocula contained  $5 \times 10^4$  to  $1.1 \times 10^7$  spores/ml of water.

Plants used for inoculation were healthy 1- to 7-month old seedlings of black and red pines grown in pots. Inoculation was carried out using a hand sprayer (Diamond Spray,

Table 1. Rates of germination, and fungal contamination of black and red pine seeds, and number of fungal isolates obtained at 25°C.

Sample (Location & lot no.)	Seeds tested (no.)	Germination <sup>1)</sup> (%)	Contamination (%)	Fungal isolates (no.)
Black pine seed :				
Mie, 79–186	150	74.0	68.7	144
Kagawa, 81–026	150	97.3	56.7	84
Red pine seed :				
Hiroshima, 81–038	150	62.0	38.7	75
Tottori, 83–073	150	73.3	100	163
Nagano, no. 13	150	58.0	74.0	103
Nagano, no. 24	250	77.0	85.2	240

1) 100–150 seeds assayed after 15 day incubation on water agar plates.

Kanemi Industry, Ltd.) (2 ml spore suspensions/plant). Inoculated plants were kept wet for 3—5 days. All experiments were conducted in a growth chamber at 26°C for 12 hr of light and 12 hr of darkness. Two to 7 plants were inoculated for each isolate in four separate experiments.

Table 2. Fungus genera and number of isolates obtained from seeds (lot numbers 79-186 and 81-026) of black pine at 10 and 25°C.

Genera	79-186			81-026			Total
	10°C	25°C	Total	10°C	25°C	Total	
Zygomycotina							
<i>Mucor</i>	1		1				1
Ascomycotina							
<i>Chaetomium</i>		3	3	1		1	4
Deuteromycotina							
<i>Alternaria</i>	1	1	2		2	2	4
<i>Arthrinium</i>	4	4	8	3	2	5	13
<i>Aspergillus</i>		4	4				4
<i>Aureobasidium</i>		1	1				1
<i>Botryodiplodia</i>					1	1	1
<i>Candida</i>	1		1				1
<i>Cephalosporium</i>				4	1	5	5
<i>Cladosporium</i>	20	25	45	7	16	23	68
<i>Curvularia</i>	2		2	1		1	3
<i>Diplodia</i> (?)		4	4				4
<i>Humicola</i> (?)				1		1	1
<i>Myrothecium</i>		2	2				2
<i>Nigrospora</i>					1	1	1
<i>Oidiodendron</i>				7	1	8	8
<i>Penicillium</i>	89	82	171	14	24	38	209
<i>Pestalotia</i>	1	8	9	9	5	14	23
<i>Phialophora</i>	2		2		1	1	3
<i>Phoma</i>	1		1		1	1	2
<i>Phomopsis</i>	1	5	6				6
<i>Pithomyces</i>				3		3	3
<i>Pyrenochaeta</i>					1	1	1
<i>Rhinoctadiella</i>				1		1	1
<i>Sporidesmium</i>				1	1	2	2
<i>Sporobolomyces</i>				1		1	1
<i>Tritirachium</i>		1	1				1
<i>Tripaspermum</i>					1	1	1
<i>Ulocladium</i>	5		5				5
<i>Verticillium</i>	1		1	1	7	8	9
UNKNOWN	6	4	10	17	19	36	46
Total	135	144	279	71	84	155	434

## Results

A total of 1,615 isolates, i. e., 434 from black pine seeds and 1,181 from red pine seeds were obtained in this study. The fungus genera and number of isolates obtained from these seeds at 10 and 25°C were summarized in Tables 2 and 3.

The fungus isolates were classified into 41 genera in which 2 genera each were Ascomy-

Table 3. Fungus genera and number of isolates obtained from seeds (lot numbers, 81-038, 83-073, nos. 13 and 24) of red pine at 10 and 25°C.

Genera	81-038			83-073			no. 13			no. 24			Total
	10°C	25°C	Total	10°C	25°C	Total	10°C	25°C	Total	10°C	25°C	Total	
Zygomycotina													
<i>Mucor</i>							2	2	4				4
<i>Syncephalastrum</i>								1	1		1	1	2
Ascomycotina													
<i>Chaetomium</i>				3	7	10	4	17	21	12	28	40	71
<i>Thielavia</i>								1	1				1
Basidiomycotina													
Unidentified					1	1				1		1	2
Deuteromycotina													
<i>Alternaria</i>		3	3		3	3	8		8				14
<i>Arthrinium</i>	11	16	27	2	3	5					1	1	33
<i>Aspergillus</i>		2	2	2		2	3	8	11		8	8	23
<i>Aureobasidium</i>	6	4	10	2		2				5	1	6	18
<i>Camposporium</i>							5		5				5
<i>Cephalosporium</i>		1	1	2		2							3
<i>Cladosporium</i>	7	8	15	9	11	20	1	1	2	6	10	16	53
<i>Diplodia</i> (?)				17	20	37							37
<i>Epicoccum</i>	1	1	2										2
<i>Fusarium</i>				2	1	3							3
<i>Gliocladium</i>					3	3							3
<i>Nigrospora</i>		1	1										1
<i>Oidiodendron</i>							5		5	4	2	6	11
<i>Paecilomyces</i>	1		1		2	2							3
<i>Penicillium</i>	29	11	40	78	65	143	64	67	131	14	30	44	358
<i>Pestalotia</i>	5	14	19	12	23	35							54
<i>Phialophora</i>	12	6	18	10		10	19		19	6		6	53
<i>Phoma</i>		4	4										4
<i>Phomopsis</i>	1	1	2	7	13	20							22
<i>Scopulariopsis</i>											2	2	2
<i>Thysanophora</i>					2	2							2
<i>Trichoderma</i>				2	7	9		2	2		1	1	12
<i>Tripospermum</i>	1		1	1		1				1	3	4	6
<i>Ulocladium</i>							1	4	5	1		1	6
UNKNOWN		3	3	2	2	4	12		12	201	153	354	373
Total	74	75	149	151	163	314	124	103	227	251	240	491	1,181

cotina and Zygomycotina, and the others, Deuteromycotina and two Basidiomycetous fungi. Among 419 unknown isolates that were not sporulated and unidentified, 358 isolates were more or less the same in colony characteristics and formation of sclerotium-like hyphal structures, and these isolates were tentatively named X<sub>1</sub> (Plate 10, Fig. 10).

The dominant fungi of these pine seeds (with isolation frequencies of more than 5%) were illustrated in relation to isolation frequency (Fig. 1).

When 7-month-old seedlings of black and red pine were inoculated by spraying with spore suspensions ( $4.1 \times 10^4 - 5 \times 10^5$  spores/ml of water) of two *Pestalotia* isolates, no conspicuous symptom developed in any inoculated plants for 15 days. Therefore, the plants were re-inoculated following the previous inoculation method. For the control, sterilized distilled water

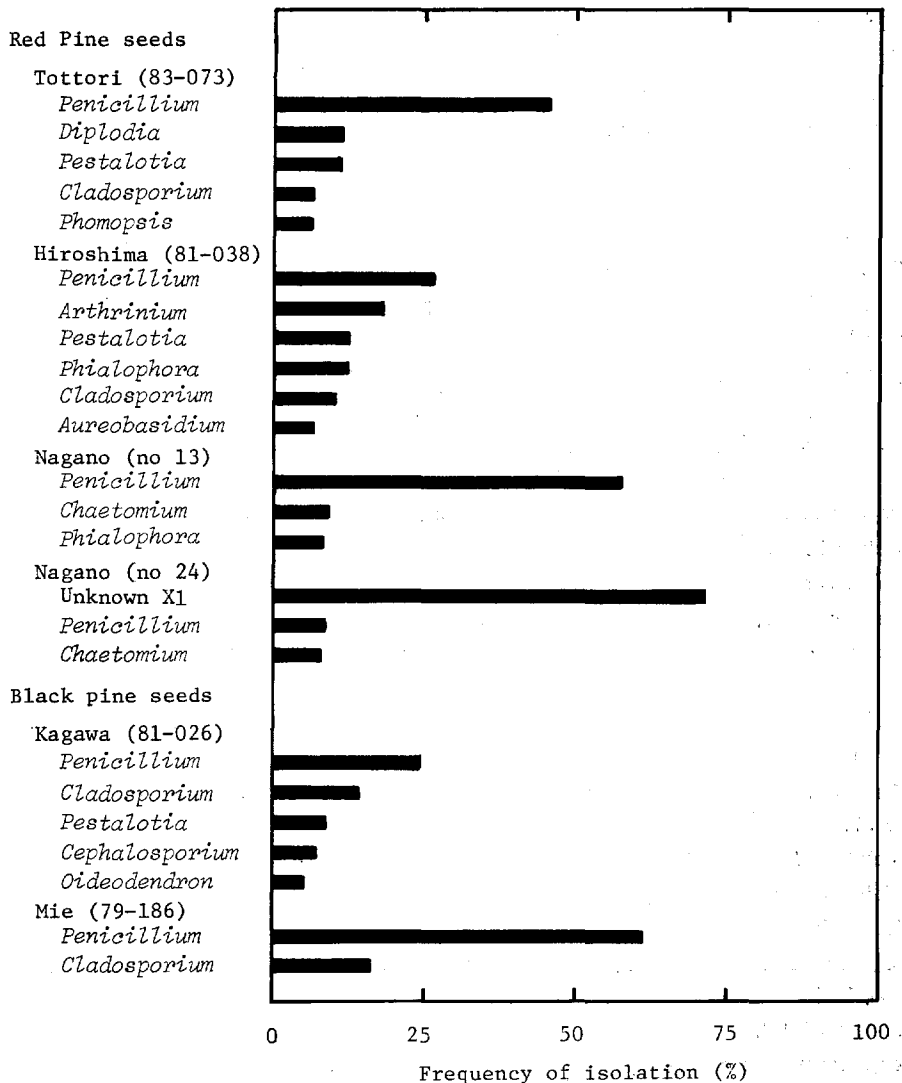


Fig. 1. Dominant fungi (with isolation frequencies of more than 5%) associated with black and red pine seeds collected from six locations in Japan.

was sprayed onto healthy seedlings.

Incidence of the disease was observed only on black pine seedlings inoculated with 83-3, black pine seed isolate 25 days after reinoculation (Plate 1, Fig. 4), but other seedlings were all healthy. In the diseased black pine seedlings, some lower needles were dead or discolored with formation of black spore masses on their basal parts. The inoculated fungus was recovered from the diseased plants.

Pathogenicity of two *Phomopsis* isolates were tested onto 1-month old seedlings of red pine. Five out of 14 seedlings inoculated with 84-517 by spraying spores ( $5.5 \times 10^6 - 1.1 \times 10^7$  spores/ml of water) collapsed completely within 30 days after inoculation, whereas none of 7 seedlings inoculated with 84-520 ( $5 \times 10^4$  spores/ml of water) and 7 uninoculated control were diseased.

Incidence of the disease started by formation of water-soaked condition of a needle 13 days after inoculation followed by wilting and shriveling of whole needles, buds, and stems, resulting in the complete collapse of the entire plants (Plate 1, Figs. 5, 6). Abundant mycelial growth on the damaged needles, formation of pycnidia, and yellow ooze of spores from pycnidia occurred on such collapsed seedlings. The fungus inoculated was always recovered from such diseased seedlings.

### Descriptions of the fungi

The fungi studied were described or explained briefly on the microscopic characteristics together with major references and photomicrographs. Colony characteristics are usually abbreviated because of space limitation.

For identification, the following references were mainly consulted: ARX (1970), BARNETT & HUNTER (1972), BARRON (1968), DOMSCH et al. (1980), ELLIS (1971, 1976), HAWKSWORTH et al. (1983) and WATANABE (1975 a, 1975 b, 1975 c). The fresh cultures and most of their exsiccati were stored in the laboratory of Forest Mycology.

***Alternaria alternata*** (FRIES) KEISSLER, in SIMMONS, Mycologia 59 : 74, 1967.

Isolate 83-22.

***Arthrinium* sp.**

Isolates 83-27, 84-469. (Plate 2, Figs. 1, 2)

Conidia characteristically lenticular with hyaline slit, brown in color, mostly  $5-7.5 \times 3-5 \mu\text{m}$ .

This fungus is close to the anamorph of *Apiospora montagnei* Sacc., but differs from the latter on the thickness of conidia.

***Aspergillus glaucus*** group, (3 types) in DOMSCH, GAMS & ANDERSON, Compendium of soil fungi Vol. 1 : 77, 1980.

Isolates 84-466; -567; -568.

Yellowish encrusted hyphae present.

Three isolates (3 types) were different in colony characteristics and conidial morphology.

***Aspergillus niger*** van TIEGHEM, in DOMSCH, GAMS & ANDERSON, Compendium of soil fungi Vol. 1 : 100, 1980.

Isolate 84-464.

***Aspergillus versicolor*** group, in DOMSCH, GAMS & ANDERSON, Compendium of soil fungi, Vol. 1 : 78, 1980.

Isolate 84-465.

Conidial heads radiating, bluish green with a grayish tint. Vesicles globose,  $12.5-17.5 \mu\text{m}$

diam, with uniseriate phialide. Conidiophores smooth-walled, pale brown, not constricted below the vesicles. Conidia globose, bluish green in mass, verrucose,  $2.1\text{--}3.6\text{ }\mu\text{m}$  diam.

*Aspergillus wentii* group, (2 types) in DOMSCH, GAMS & ANDERSON, Compendium of soil fungi, Vol. 1: 122, 1980.

Isolates 84-463; -467. (Plate 2, Fig. 3)

Two isolates (2 types) were different in colony characteristics and morphology of conidiophores and conidia. In 84-463, conidiophores are colorless and conidia are globose or subglobose, slightly verrucose,  $4.5\text{--}6.3\text{ }\mu\text{m}$  diam, whereas in 84-467, conidiophores are brown, and conidia are various in shape, globose to elliptical,  $3.6\text{--}5.1\text{ }\mu\text{m}$  in long diam, conspicuously verrucose.

*Aureobasidium pullulans* (DE BARY) ARNAUD, in ELLIS, Dematiaceous Hyphomycetes, p. 515, 1971.

Isolates 83-50, 84-473; -474. (Plate 2, Fig. 4)

Conidia directly borne on hyphae singly or in mass, hyaline or subhyaline, cylindrical, usually 1-celled, but rarely septate, various in size, mostly  $6.2\text{--}12.5 \times 2.5\text{--}3.8\text{ }\mu\text{m}$ .

**Basidiomycetous fungi** (2 types)

Isolates 84-481; -595. (Plate 2, Figs. 5, 6)

Two isolates (2 types) were different in colony characteristics; isolate 84-484 had white colony with localized brown patches, whereas 84-595, yellowish brown colony.

**Botryodiplodia** sp.

Isolate 84-476. (Plate 2, Figs. 7, 8)

Pycnidia formed usually in aggregate, irregular in shape, dark brown, with abundant exudation of spore mass. Conidia mostly aseptate, occasionally 1-septate, hyaline or subhyaline,  $7.5\text{--}17.5 \times 2.2\text{--}4.5\text{ }\mu\text{m}$ .

**Camposporium** sp.

Isolate 84-581. (Plate 3, Figs. 1, 2)

Conidia borne singly and apically on or near the abruptly-tapered apex of simple conidiophores, cylindrical, pale brown, 1- to 3-septate, mostly  $12.5\text{--}15.3 \times 5\text{--}6\text{ }\mu\text{m}$ . Conidiophores cylindrical, 1-3-septate, yellowish brown, often constricted at base,  $15\text{--}37.5 \times 2.7\text{--}3.6\text{ }\mu\text{m}$ .

This fungus is different from previously-described species of *Camposporium* (HUGHES 1951, ICHINOE 1971) on the basis of the number of septation, size, and absence of appendages of conidia, and it appears to be a new species.

**Candida** spp. (2 types)

Isolates 83-87, 84-569. (Plate 3, Fig. 3)

Conidia borne singly or in aggregate directly on hyphae, characteristically propagated by budding, hyaline, 1-celled.

Isolate 83-87 had white colony and its conidia did not contain eye spot, whereas 84-569 had pinkish colony and its conidia were with 2 eye spots and narrower in width.

**Cephalosporium** (= *Acremonium*) spp. (2 types)

Isolates 84-478; -479. (Plate 3, Fig. 4)

Two isolates (2 types) were different in colony characteristics and other morphology.

Note that the genus name *Acremonium* is more accepted than *Cephalosporium*.

**Chaetomium africanum** AMES, in A monograph of the Chaetomiaceae, p. 10, 1969.

Isolates 84-490; -573.

Perithecia subglobose, with well developed rhizoids,  $175\text{--}250\text{ }\mu\text{m}$  diam. Terminal hairs various, dichotomously branched with short or long elongation, twisted, irregularly branched

or occasionally stiff, straight, crustaceous. Asci clavate,  $20-37.5 \times 6.2-10 \mu\text{m}$ . Ascospores ovate, non-apiculate,  $5-6.3 \times 2.8-4 \mu\text{m}$ .

***Chaetomium cochlioides*** PALLISER, in AMES, A monograph of the Chaetomiaceae, p. 17, 1969.

Isolate 84-487.

Perithecia subglobose or barrel-shaped,  $250-275 \times 190-225 \mu\text{m}$ . Terminal hairs spirally coiled or undulate, yellowish brown. Asci clavate,  $110-135 \times 20-25 \mu\text{m}$ . Ascospores lemon-shaped, apiculate at both ends,  $7.5-8.6 \times 7.2-7.5 \mu\text{m}$ .

***Chaetomium dolichotrichum*** AMES, in Mycologia 37 : 145, 1945.

Isolates 83-26, 84-101; -483; -494. (Plate 3, Figs. 5, 6)

Perithecia subglobose,  $75-113 \mu\text{m}$  diam. Terminal hairs dichotomously branched or straight. Asci clavate,  $20-37.5 \times 6.2-8.8 \mu\text{m}$ . Ascospores ovate or elliptical, slightly apiculate, pale brown,  $5.5-7.3 \times 2.5-4.5 \mu\text{m}$ .

***Chaetomium erectum*** SKOLKO & GROVES, in AMES, A monograph of the Chaetomiaceae, p. 23, 1969.

Isolate 84-489.

Perithecia subglobose, dark green,  $125-150 \mu\text{m}$  diam. Terminal hairs dichotomously branched, occasionally straight, especially stiff when immature. Asci, club-shaped. Ascospores ovate, subapiculate at one end, brown,  $5.2-5.5 \times 3.7-4.3 \mu\text{m}$ .

***Chaetomium funiculum*** COOKE, in AMES, A monograph of the Chaetomiaceae, p. 24, 1969.

Isolate 84-484.

Perithecia subglobose, with rhizoid,  $110-140 \mu\text{m}$  diam. Terminal hairs, dichotomously branched or straight, stiff. Asci club-shaped,  $20-27.5 \times 7-7.5 \mu\text{m}$ . Ascospores lemon-shaped, broadly ovate, apiculate well at one end, pale brown,  $5-5.5 \times 3-3.9 \mu\text{m}$ .

***Chaetomium globosum*** KUNZE, in AMES, A monograph of the Chaetomiaceae, p. 26, 1969.

Isolates 83-25, 84-485; -491; -493. (Plate 7, Figs. 7, 8)

Perithecia subglobose,  $125-215 \mu\text{m}$  diam. Terminal hairs undulate. Asci cylindro-clavate,  $50-88 \times 10-12.5 \mu\text{m}$ . Ascospores lemon-shaped, subglobose, apiculate at both ends, brown,  $8.7-10 \times 7.5-9.5 \mu\text{m}$ .

***Chaetomium reflexum*** SKOLKO & GROVES, in Can. J. Res. C, 26 : 279, 1948.

Isolate 84-488, (Plate 3, Figs. 9, 10)

Perithecia subglobose,  $40-100 \mu\text{m}$  diam. Terminal hairs dichotomously branched, strongly recurved. Asci club-shaped, nearly  $20 \times 20 \mu\text{m}$ . Ascospores ovate, subapiculate at both ends, brown,  $4.5-6.3 \times 2.7-3.8 \mu\text{m}$ .

***Chaetomium* sp.**

Isolate 84-492.

Perithecia with unbranched, but occasionally forked terminal hairs.

This fungus is close to *C. gracile* UDAGAWA, but its ascospores are smaller in size than those of the latter fungus.

***Cladosporium cladosporioides*** (FRESNIUS) de VRIES, in Contribution to the knowledge of the genus *Cladosporium* LINK ex FR. p. 57, 1952.

Isolate 83-46.

***Curvularia senegalensis*** (SPEG) C. SUBRAMANIAN, in Ellis, Mycol. Pap. 106 : 37, 1966.

Isolate 84-502. (Plate 4, Fig. 1)

Conidia typically 4-septate, smooth-walled, without hilum, brown,  $20-32.5 \times 7.5-11.3 \mu\text{m}$ , with darker central cells than the rest.



**Diplodia sp.**

Isolates 83-75, 84-496; -497; -500.

None of the isolates tested did sporulate in various media including PDA 20 days after plating. VERRALL (1942) pointed out that isolates of *D. natalensis* from logs and lumber were difficult to induce fruiting.

**Epicoccum purpurascens** EHRENB. ex SCHLECHT, in DOMSCH, GAMS & ANDERSON, Compendium of soil fungi p. 279, 1980.

Isolate 84-526. (Plate 4, Figs. 2, 3)

Conidia globose or subglobose, smooth or verrucose, borne on directly on hyphae, muriform, often with a protuberant basal cell, golden brown to dark brown, mostly 15–20  $\mu\text{m}$  diam.

**Fusarium moniliforme** (SHELD.) emend. SNYDER & HANSEN, in Amer. J. Bot. 32 : 664, 1945.

Isolate 84-503.

**Gliocladium roseum** BAINIER, in DOMSCH, GAMS, & ANDERSON, Compendium of soil fungi Vol. 1 : 371, 1980.

Isolates 84-544; -545. (Plate 4, Figs. 4, 5)

Conidiophores of two kinds; verticillate and penicillate. Conidia cylindrical to subcylindrical, asymmetrically navicular, hyaline, 4.3–9  $\times$  1.7–3.6  $\mu\text{m}$ .

**Humicola sp.**

Isolate 84-582. (Plate 4, Fig. 6)

Conidia formed in aggregate, borne on sterigmata directly on hyphae, subglobose, smooth, brown, apiculate or truncate at one end, thick-walled, 4.8–5.4  $\times$  3.6–4.5  $\mu\text{m}$ .

This fungus is different from previously-described species of *Humicola* on conidial size and it appears to be a new species.

**Mucor plumbeus** BONORD, in DOMSCH, GAMS & ANDERSON, Compendium of soil fungi Vol. 1 : 475, 1980.

Isolate 84-508. (Plate 4, Figs. 7, 8)

Sporangia subglobose, black, spinulose, up to 100  $\mu\text{m}$  diam. Sporangiophores more than 2 mm long, 15–25  $\mu\text{m}$  wide at base. Columella cylindrical, characteristically with a few projections to 5  $\mu\text{m}$  long, pale brown, mostly 36–40  $\times$  20–22.5  $\mu\text{m}$ . Sporangiospores globose, pale brown, mostly 5–7.5  $\mu\text{m}$  diam. Chlamydospores globose, subglobose or cylindrical, yellowish, mostly 17.5–30  $\times$  15–20  $\mu\text{m}$ .

**Myrothecium verrucaria** (ALB. & SCHWEIN.) DITMAR ex FR., in PRESTON, Trans. Brit. mycol. Soc. 26 : 167, 1943; Tulloch, Mycol. Pap. 130 : 27, 1972.

Isolate 83-44; -45. (Plate 5, Figs. 1, 2)

Sporodochia discoid, black, without setae, less than 1 mm diam. Conidia elliptical, truncate at one end, hyaline to subhyaline, smooth, mostly 5.4–6.5  $\times$  1.8–2.5  $\mu\text{m}$ .

**Nigrospora sacchari** (SPEG.) MASON, in ELLIS, Dematiaceous Hyphomycetes p. 320, 1971.

Isolate 84-509. (Plate 5, Fig. 3)

Conidia black, 15–25  $\times$  12.5–17.5  $\mu\text{m}$ .

**Nigrospora sp.** (anamorph of *Khuskia oryzae* HUDSON), in ELLIS, Dematiaceous Hyphomycetes, p. 320, 1971.

Isolate 83-63.

Conidia small, black, 11.2–13.8  $\times$  9.5–10  $\mu\text{m}$ .

**Oidiodendron cerealis** (THUM) BARRON, in Can. J. Bot. 40 : 594, 1962.

Isolates 84-506; -507; -572. (Plate 5, Figs. 4, 5)

Conidia formed in chains, characteristically lens-shaped, ovoid, or subglobose, brown, double-walled, smooth,  $2.1-4.5 \times 1.8-2 \mu\text{m}$ .

**Oidiendron flavum** SZILVINYI, in BARRON, Can. J. Bot. 40 : 598, 1962.

Isolates 84-475; -570; -571; -597; -610. (Plate 5, Figs. 6, 7)

Colonies on PDA, limited in growth, less than 30 mm diam in 15 days, pale mustard yellow, yellowish green or yellowish brown. Conidia globose, ovoid, lemon-shaped, often angular, various in shape, hyaline, mostly  $1.8-3.3 \mu\text{m}$ .

**Paecilomyces parvus** BROWN & SMITH, in Trans. Br. mycol. Soc. 40 : 58, 1957.

Isolate 84-596.

Conidial heads mostly simple. Phialide swollen up to one half in length from base, nearly  $10 \mu\text{m}$  long,  $1.6 \mu\text{m}$  wide. Conidia subglobose,  $2.5-3.3 \mu\text{m}$  diam.

**Paecilomyces roseolus** SMITH, in Trans. Br. mycol. Soc. 45 : 388, 1962; Onions and Barron, Mycol. Pap. 107 : 8, 1967.

Isolate 84-510. (Plate 5, Figs. 8, 9)

Phialides usually solitary,  $12.5-30 \mu\text{m}$  long,  $2.5 \mu\text{m}$  diam, tapering gradually from base to apex, mostly developed from funicular hyphae. Conidia formed in chains, lemon-shaped, hyaline,  $5.2-7 \times 2.5-2.8 \mu\text{m}$ .

**Penicillium spp.** (16 types or more)

Isolates 84-547-84-566.

At least 16 types were differentiated to one another mainly based on colony characteristics and conidial morphology.

**Pestalotia spp.** (4 types or more)

Isolates 83-1; -2; -3; -5; 84-511; -512; -513. (Plate 6, Figs. 1-4)

At least 4 types were differentiated to one another on the basis of colony characteristics and conidial morphology. For example, conidia have very short appendages in Type I (Isolate 83-1), conspicuously dark central cells in Type II (83-2), or cylindrical shape with constriction at each septum in Type III (83-3), and one conspicuously thick and dark central septum in Type IV (83-5).

**Phialophora atrovirens** (BEYMA) SCHOL-SCHWARZ, in Persoonia 6 : 65, 1970.

Isolate 84-524. (Plate 6, Figs. 5, 6)

Colony less than 2 cm diam in 20 days on PDA, dark brown, with pale brown pigment produced in the margin of colony, raised centrally and clear in the margin. Phialide borne singly or in aggregate on hyphae or on conidiophores, hyaline or subhyaline, corpulent, inflated in the middle and constricted at base, mostly  $4.5-11 \times 2-3.5 \mu\text{m}$ . Conidia globose, hyaline, guttulate, mostly  $1.7-4.5 \mu\text{m}$  diam. Hyphae heavily globulate. Chlamydospore-like structures formed in chain terminally or intercalary,  $3.7-8.8 \mu\text{m}$  in each cell, often brown, verrucose after maturity.

This fungus is close to *P. cyclaminis* BEYMA, Group B with shorter swollen phialides and clusters of swollen cells described by GAMS and DOMSCH (1969).

**Phialophora fastigata** (LAGERBERG & MELIN) CONANT, in SCHOL-SCHWARZ, Persoonia 6 : 71, 1970;

COLE and KENDRICK, Mycologia 65 : 677, 1973.

Isolates 84-574; -604. (Plate 6, Fig. 10)

Phialides borne singly or as part of conidiophores, brownish, occasionally septate, with collarette that is not conspicuous when immature. Conidia ovoid, hyaline or subhyaline, mostly  $3.7-6 \times 2-3 \mu\text{m}$ .

**Phialophora malorum** (KIDD & BEAUMONT) MCCOLLOCH, in SCHOL-SCHWARZ, Persoonia 6 : 75, 1970.

Isolate 83-37. (Plate 6, Figs. 7—9)

Phialide with collarette that is not conspicuous when immature, but clearer with age. Conidia ovoid, elliptical, hyaline,  $6.3-12.6 \times 2-2.2 \mu\text{m}$ .

*Phoma glomerata* (CORDA) WOLLENW. & HOCHAPP., in DORENBOSCH, Persoonia 6 : 7, 1970.

Isolate 83-17.

Dictyochlamydospores formed singly or in chains, usually  $15-42.5 \times 10-20 \mu\text{m}$ . Pycnidia nearly  $50 \mu\text{m}$  diam with single apical ostiole. Conidia elliptical, with 2 eye spots, nearly  $5 \times 1.8 \mu\text{m}$ .

*Phoma* sp.

Isolate 84-515.

Pycnidia brown, with apical ostiole,  $75-150 \mu\text{m}$  diam. Conidia cylindrical, hyaline,  $3-3.8 \times 1.2-1.8 \mu\text{m}$ .

*Phomopsis* spp. (2 types or more)

Isolates 83-9, 84-517. (Plate 7, Fig. 1)

At least two types were differentiated on the basis of colony characteristics and conidial morphology. In Type I (Isolate 83-9), alpha spores, elliptical, hyaline,  $6.3-10.8 \times 1.8 \mu\text{m}$  and beta spores more than  $27 \mu\text{m}$  long, whereas in Type II (Isolate 84-517), alpha spores, cylindrical,  $5.7-7.2 \times 1.8-2.7 \mu\text{m}$  and beta spores, usually  $20-33 \mu\text{m}$  long.

*Pithomyces chartarum* (BERK. & CURT) M. B. ELLIS, in Myco. Pap. 76 : 13, 1960.

Isolate 84-585. (Plate 7, Fig. 2)

Conidia borne singly on simple conidiophores, broadly oval, brown, very rarely verrucose, usually with 3 transverse septa and 1—2 longitudinal septa,  $21.2-25 \times 12.5-15 \mu\text{m}$ .

Note : Conidia are mostly smooth, but very rarely verrucose.

*Pithomyces maydis* (SACC.) M. B. ELLIS, in Mycol. Pap. 76 : 15, 1960.

Isolate 84-516. (Plate 7, Fig. 3)

Conidia borne singly and apically on simple conidiophores, broadly ellipsoid, brown, 2-septate transversely,  $20-23.4 \times 12.5-12.6 \mu\text{m}$ .

*Pyrenochaeta* sp.

Isolate 84-523. (Plate 7, Figs. 4, 5)

Pycnidia barrel-shaped or subglobose, yellowish brown, with characteristically short setae around the ostiolar region, mostly  $50-87.5 \times 57.5-72.5 \mu\text{m}$ . Setae dark brown, thick-walled, tapering toward end, not sharp,  $7.5-17.5 \mu\text{m}$  long,  $4.5-5.4 \mu\text{m}$  wide at base. Conidia globose hyaline, angular,  $1.8-2.4 \mu\text{m}$  diam. This fungus appears to be a new species.

*Rhinocladiella anceps* (SACC. et ELLIS) HUGHES, in SCHOL-SCHWARZ, Antonie van Leeuwenhoek 34 : 143, 1968.

Isolate 84-525. (Plate 7, Figs. 6, 7)

Conidia borne apically and laterally on fertile denticulate portion of simple conidiophores, lacrymoid,  $3.6-6.5 \times 1.6-4.5 \mu\text{m}$ . Conidiophores gradually tapering from base to apex,  $160-235 \mu\text{m}$  long,  $1.6-3.3 \mu\text{m}$  wide at base.

*Scopulariopsis brevicaulis* (SACC.) BAINIER, in MORTON & SMITH, Mycol. Pap. 86 : 39, 1963.

Isolate 84-577. (Plate 7, Fig. 10)

Conidiophores cylindrical, mostly  $9-16.2 \times 2.7-3.6 \mu\text{m}$ . Conidia globose or subglobose, with truncate base, pale yellowish brown, verrucose,  $5.4-8.1 \mu\text{m}$  diam.

*Scopulariopsis canadensis* MORTON & SMITH, in Mycol. Pap. 86 : 55, 1963.

Isolate 84-514. (Plate 7, Figs. 8, 9)

Annellophores more or less cylindrical, often curved, hyaline,  $25-50 \times 2.5-2.8 \mu\text{m}$ . Conidia catenulate, ovate, subglobose, truncate at one end, hyaline, smooth, mostly  $5-8.1 \times 3.5-5.4 \mu\text{m}$ .

***Sporidesmium* sp.**

Isolate 84-578. (Plate 8, Figs. 1, 2)

Conidia borne apically on simple conidiophores, elliptical, ovate, or cylindrical, thick-walled, golden-brown to dark brown, 1-3-septate, mostly 2-septate, constricted occasionally at both sides of the first septum near conidiophore, deciduous with or without pedicel,  $16.2-35 \times 7.5-12.5 \mu\text{m}$ . Conidiophores simple,  $5-27.5 \times 1.8-2.2 \mu\text{m}$ . This fungus appears to be a new species.

***Sporobolomyces* sp.**

Isolate 84-578. (Plate 8, Figs. 3, 4)

Colony yellowish white. Conidia shot off violently, cylindrical, sickle-shaped, hyaline, mostly  $7.2-12.6 \times 1.5-2.5 \mu\text{m}$ . Hyphae without clamp connexion,  $1.8-2.7 \mu\text{m}$  wide.

***Synecephalastrum racemosum*** COHN ex SCHROT, in DOMSCH, GAMS & ANDERSON, Compendium of soil fungi Vol. 1 : 751, 1980.

Isolate 84-529. (Plate 8, Figs. 5-7)

Spore heads up to  $110 \mu\text{m}$  diam, black, composed of merosporangia and vesicles borne apically on sporangiophores. Sporangioophores branched, with rhizoids, more than 3 mm tall, pale brown. Vesicles globose, with tiny holes all over,  $25-50 \mu\text{m}$  diam, larger on the main sporangiophores than on branches. Merosporangia mostly 3-9 sporangiospores per merosporangium,  $8.7-27.5 \mu\text{m}$  long. Sporangiospores globose, subglobose or occasionally cuneiform, smooth, hyaline, mostly  $3.6-6.3 \mu\text{m}$  diam.

***Thielavia terricola*** (GILMAN & ABBOTT) EMMONE, in MALLOCH and CAIN, Mycologia 65 : 1071, 1973.

Isolate 84-532. (Plate 8, Fig. 8)

Ascarps globose, dark brown, glabrous, mostly  $100-115 \mu\text{m}$  diam, with a thin semi-transparent wall. Asci clavate, pedicelate, mostly  $27.5-35 \times 13.7-17.5 \mu\text{m}$ , 8-spored. Ascospores elliptical, truncate at one end, dark brown in mass,  $12.2-13.8 \times 5.5-7.5 \mu\text{m}$ . Conidia not formed.

***Thysanophora penicilloides*** (ROUMEGUERE) KENDRICK, in Can. J. Bot. 39 : 820, 1961.

Isolate 84-531. (Plate 9, Figs. 1, 2)

Penicilli biverticillate, borne on well developed conidiophores. Conidia borne in chains, subglobose, hyaline or subhyaline,  $1.8-2.2 \mu\text{m}$  diam. Conidiophores 10-16-septate, dark brown,  $500-600 \mu\text{m}$  tall,  $7.5 \mu\text{m}$  diam. Sclerotia globose, black, nearly  $240 \mu\text{m}$  diam.

***Trichoderma harizianum*** RIFAI, in Mycol. Pap. 116 : 38, 1969.

Isolate 84-530. (Plate 3, Fig. 3)

Conidia globose or subglobose, smooth, pale green,  $2.1-2.7 \mu\text{m}$  diam. Chlamydospores borne intercalary, mostly  $7-8 \mu\text{m}$  diam. Needle-shaped crystals abundantly formed, golden yellow.

This isolate is close to *T. aureoviride* RIFAI on the basis of formation of crystals, but different from it in conidial morphology.

***Triperspermum myrti*** (LIND.) HUGHES, in Mycol. Pap. 46 : 18, 1951.

Isolate 84-533. (Plate 9, Figs. 4, 5)

Conidia composed of stalked cell and 4-armed cells. Armed cells 3-4-celled, tapering toward end,  $17.5-27 \mu\text{m}$  long. Stalked cell 1-2-celled,  $6.2-10 \mu\text{m}$  diam.

***Tritirachium* sp.**

Isolate 83-16. (Plate 9, Figs. 6-9)

Conidiophores well developed, often densely verticillate with apical sterile elongation. Conidia produced on a symposula, ovate, subglobose, often apiculate at one end, hyaline,  $2.1-$

3.8×1.8—2.5  $\mu$ m.

***Ulocladium chartarum*** SIMMONS, in Mycologia 59 : 88, 1967.

Isolate 84-505. (Plate 10, Figs. 1, 2)

Conidia borne in chains, acropetally-developed, muriform, brown, often verrucose, mostly 21.2—31.3×12.5—20  $\mu$ m, occasionally with one or two beaked cells : 5—17.5×3—3.8  $\mu$ m.

***Ulocladium oudemansii*** SIMMONS, in Mycologia 59 : 86, 1967.

Isolates 84—580; —600. (Plate 10, Fig. 10)

Conidia borne singly or occasionally in short chains on simple conidiophores apically or laterally, globose, subglobose, ellipsoidal or ovoid, yellowish brown when young, dark brown with age, muriform, usually up to 8-celled, mostly 20—37.5×12.5—20  $\mu$ m.

***Verticillium lecanii*** (ZIMM.) VIEGAS, in Domsch, GAMS & ANDERSON, Compendium of soil fungi, Vol. 1 : 840, 1980.

Isolate 84-546. (Plate 10, Fig. 4)

Colony white, yellowish in reverse on PDA. Conidiophores erect or prostrate, with verticillate phialides. Phialides gradually tapering from base to apex, 11.2—30  $\mu$ m long, 1.7—2.5  $\mu$ m at base. Conidia cylindrical with rounded tips, or ellipsoidal, 3.7—10×0.7—1.7  $\mu$ m.

***Verticillium* sp.**

Isolate 84-477. (Plate 10, Figs. 5—9)

Conidiophores verticillate or sympodially developed, hyaline, 1-septate at or near the branching part, with 2 to several sporogenous cells and the conidial heads of 7.5—15  $\mu$ m diam at their apices. Sporogenous cells tapering gradually from the branching part nearly 2.5  $\mu$ m wide toward apex 1.8  $\mu$ m wide, 1-septate in the central part, mostly 125—250  $\mu$ m long. Conidia borne in conidial heads, globose, slightly angular, hyaline, 1.4—2  $\mu$ m diam. Chlamydospores borne terminal or intercalary, globose, cylindrical, ovate, double-walled, pale yellow, often with septum development, 32.5—40  $\mu$ m diam in globose chlamydospores, 47.5—82.5×35—42.5  $\mu$ m for cylindrical ones.

This fungus is similar to *Mortierella* and may be a new species, although it has septate hyphae.

**X<sub>1</sub>**, Unidentified

Isolate 84-615; —616. (Plate 10, Fig. 10)

Sclerotium-like structures formed characteristically, nearly 50  $\mu$ m diam. No other morphologically characteristic structures observed.

### Discussion

Many fungi associated with pine seeds including *Penicillium* and *Chaetomium* spp. sporulated directly on the seeds plated on agar media within 2—10 days after treatment (Plate 1, Figs. 1—3). Therefore, some fungi may be easily identified without pure isolation. However, not many fungi could be differentiated or identified correctly from one another by direct observation of the seeds.

For identification, the morphology of organs formed in nature is very important, especially for parasitic fungi in the modern taxonomy. For example, some *Pestalotia* isolates were identified to the species level based on morphology of conidia and pustules formed in nature. Therefore, our isolates of *Pestalotia* could not be identified to the species level because ours were obtained by single hyphal tipplings from randomly selected hyphae grown out of the seeds in which no pustules were formed.

In this study, we tried to isolate the fungi associated with seeds as many genera as possible. Therefore, the seeds to be tested were just washed well, air-dried and plated on water agar without surface sterilization. Isolations were conducted by single hyphal tipplings from randomly selected hyphae grown out of seeds.

A total of 1,615 isolates from black and red pine seeds collected from 6 different locations in Japan were classified into nearly 53 species of 41 genera. The number of genera found in this study was greater than in most of the previous studies in which the number of genera recovered was usually less than 15.

The smaller number of genera recovered in the previous studies was probably due to the following factors:

- 1) Sample scale was small, and some 100 seeds were assayed in each study.
- 2) Seeds tested were surface sterilized with chemicals such as mercury chloride, and most of the seed-born fungi were, therefore, killed.
- 3) Seeds were plated on rich media such as potato dextrose agar for the assay of the fungi. The rich media are often easily colonized by a few fast-growing fungi, and only a few species recovered in such media.

In this study, 19 or 21 genera were found associated with each lot in black pine seeds, and 13 to 19 genera in red pine seeds.

From two lots of black pine seeds, the following 10 genera were commonly found: *Alternaria*, *Arthrinium*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Penicillium*, *Pestalotia*, *Phialophora*, and *Phoma* in alphabetical order (Table 2). Except for *Cephalosporium*, *Curvularia*, *Pestalotia* and *Phoma*, the rest of the previous fungi were also found in more than 3 lots of red pine seeds (Table 3). In addition, *Aspergillus*, *Aureobasidium*, *Trichoderma* and *Tripaspermum* were also common on red pine seeds. Most of these fungi are also common as members of fungus floras associated with other seeds (SATO 1955, WATANABE 1972), and so-called field fungi because they invade seeds in the field before being harvested (CHRISTENSEN & KAUFMAN 1965). However, it is interesting to note that two Basidiomycetous isolates were obtained from red pine seeds. In addition, the common occurrence of *Pestalotia* and *Phialophora* may be characteristic for the seeds of *Pinus* and other forest trees (WATANABE, unpublished).

The dominant fungus in every seed lot was *Penicillium* except for seed lot no. 24 in which the unidentified  $X_1$  was predominant (Fig. 1).

Further work is needed for the identification of  $X_1$  and other unknown fungi. However, the results of this study may be significant as the first record on the fungus flora associated with black and red pine seeds in Japan.

The numbers of genera of fungi isolated at 7° or 10° and 25°C were almost equal, but the genera recovered under both temperature conditions were often different. For example, from seed lot 81-026 of black pine seeds, 14 genera each were recovered under both temperature conditions, but a total of 21 genera were recovered from this seed lot. Therefore, it is essential to isolate fungi under different temperature conditions for the study of fungus genera.

In the inoculation experiments, one isolate each of *Pestalotia* and *Phomopsis* was positively proved to be pathogenic to pine seedlings. Therefore, among our isolates obtained in this study, some other plant pathogens may be included, and therefore, further work on pathogenicity is needed to clarify the significance of seed-borne plant pathogens.

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### Legend for figures

#### Plate 1.

- Fig. 1. *Penicillium* sp. sporulated on a red pine seed, ( $\times 17$ ).
- Figs. 2, 3. *Chaetomium* spp. on germinated black pine (2) and ungerminated red pine seeds (3), (2 :  $\times 8$ ; 3 :  $\times 15$ ).
- Fig. 4. Pathogenicity of *Pestalotia* sp. (Isolate 83-3) on to black pine seedlings (right : inoculated; left : uninoculated) ( $\times 0.8$ ).
- Figs. 5, 6. Pathogenicity of *Phomopsis* sp. (Isolate 84-517) on to red pine seedlings. Note one needle shrivelled initially (5) and an entire plant collapsed (6), (5 :  $\times 1.3$ ; 6 :  $\times 5$ ).

#### Plate 2.

- Figs. 1, 2. *Arthrimum* sp. Hypha (1) and conidia (1, 2) (scales : 1, 2=10  $\mu$ m).
- Fig. 3. *Aspergillus* sp. (*A. wentii* group). Conidiophore and conidia (scale : 3=5  $\mu$ m).
- Fig. 4. *Aureobasidium pullulans*. Hyphae and conidia (scale : 4=5  $\mu$ m).
- Figs. 5, 6. Basidiomycetous fungi. Isolates 84-481 (5) and 84-597 (6) (scales : 5, 6=5  $\mu$ m).
- Figs. 7, 8. *Botryodiplodia* sp. Pycnidia (7) and conidia (8) (scales : 7=75  $\mu$ m; 8=10  $\mu$ m).

#### Plate 3.

- Figs. 1, 2. *Camposporium* sp. Conidiophores (1, 2) and conidia (1) (scales : 1, 2=5  $\mu$ m).
- Fig. 3. *Candida* sp. Hypha and conidia (scale : 3=5  $\mu$ m).
- Fig. 4. *Cephalosporium* sp. Habit (scale : 4=10  $\mu$ m).
- Figs. 5—10. *Chaetomium* spp. Perithecia, asci, and ascospores of *C. dolichotrichum* (5, 6), *C. globosum* (7, 8) and *C. reflexum* (9, 10) (scales : 5=20  $\mu$ m; 6, 10=5  $\mu$ m; 8, 9=10  $\mu$ m; 7=50  $\mu$ m).

#### Plate 4.

- Fig. 1. *Curvularia senegalensis*. Conidiophores and conidia (scale : 1=10  $\mu$ m).
- Figs. 2, 3. *Epicoccum purpurascens*. Conidia (2, 3) and hyphae (2) (scales : 2=10  $\mu$ m; 3=5  $\mu$ m).
- Figs. 4, 5. *Gliocladium roseum*. Habit (4), conidiophores and conidia (5) (scales : 4, 5=5  $\mu$ m).
- Fig. 6. *Humicola* sp. (?). Hyphae and conidia (scale : 6=5  $\mu$ m).
- Figs. 7, 8. *Mucor plumbeus*. Columella (7), sporangiophores and sporangiospores (8) (scales : 7=10  $\mu$ m; 8=20  $\mu$ m).

#### Plate 5.

- Figs. 1, 2. *Myrothecium verrucaria*. Sporodochium (1), conidiophores and conidia (2)



(scales : 1=20  $\mu$ m ; 2=5  $\mu$ m).

Fig. 3. *Nigrospora sacchari*. Hyphae and conidia (scale : 3=10  $\mu$ m).

Figs. 4—7. *Oidiodendron* spp. Habit, conidiophores and conidia of *O. cerealis* (4, 5) and *O. flavum* (6, 7) (scales : 4, 6=5  $\mu$ m ; 5=3  $\mu$ m ; 7=2.5  $\mu$ m).

Figs. 8, 9. *Paecilomyces roseolus*. Conidiophore with a chain of conidia (8) and conidia (9) (scales : 8, 9=5  $\mu$ m).

#### Plate 6.

Figs. 1—4. *Pestalotia* spp. Conidia of isolates 83-1 (1), 83-2 (2), 83-3 (3) and 83-5 (4) (scales : 1—4=10  $\mu$ m).

Figs. 5—10. *Phialophora* spp. Conidiophores (5, 6), conidia and globose chlamydospore-like cells (6) of *P. atrovirens*; Habit (7), conidia (8) and immature (8) and mature (9) conidiophores of *P. malorum*; Conidia and conidiophores of *P. fastigata* (10) (scales : 5—7=5  $\mu$ m ; 8, 9=3  $\mu$ m ; 10=10  $\mu$ m).

#### Plate 7.

Fig. 1. *Phomopsis* sp. Alpha and beta conidia of Isolate 83-9 (scale : 1=5  $\mu$ m).

Figs. 2, 3. *Pithomyces* spp. Hyphae and conidia of *P. chartarum* (2) and *P. maydicus* (3) (scales : 2, 3=10  $\mu$ m).

Figs. 4, 5. *Pyrenochaeta* sp. Conidia and pycnidia (4, 5) with setae around the ostiolar region (5) (scales : 4, 5=10  $\mu$ m).

Figs. 6, 7. *Rhinoctadiella anceps*. Habit (7) and conidiophores and conidia (6) (scales : 6=5  $\mu$ m ; 7=10  $\mu$ m).

Figs. 8—10. *Scopulariopsis* spp. Habit (8) and conidiophores and conidia of *S. canadensis* (9); Conidiophore and conidia of *S. bravicaulis* (10) (scales : 8=10  $\mu$ m ; 9, 10=5  $\mu$ m).

#### Plate 8.

Figs. 1, 2. *Sporidesmium* sp. Conidia (1, 2) and hyphae (1) (scales : 1, 2=10  $\mu$ m).

Figs. 3, 4. *Sporobolomyces* sp. Hyphae, conidia and budding cells (scales : 3=2  $\mu$ m ; 4=5  $\mu$ m).

Figs. 5—7. *Syncephalastrum racemosum*. Sporangioophores (5, 7) heads of spores (5), vesicles (7) and sporangiospores (6, 7) (scales : 5, 7=20  $\mu$ m ; 6=5  $\mu$ m).

Fig. 8. *Thielavia terricola*. Ascocarp crushed, asci and ascospores (scale : 8=20  $\mu$ m).

#### Plate 9.

Figs. 1, 2. *Thysanophora penicilloides*. Habit (1), and conidiophores, conidial heads and conidia (2) (scales : 1=20  $\mu$ m ; 2=3  $\mu$ m).

Fig. 3. *Trichoderma harizianum*. Conidia and needle-shaped crystals (scale : 3=2.5  $\mu$ m).

Figs. 4, 5. *Tripospermum myrti*. Conidia (4, 5) and hyphae (5) (scales : 4, 5=10  $\mu$ m).

Figs. 6—9. *Tritirachium* sp. Habit (6, 7), conidiophores and conidia (8, 9) (scales : 6—9=5  $\mu$ m).

#### Plate 10.

Figs. 1—3. *Ulocladium* spp. Habit (1), conidiophores and conidia of *U. chartarum* (2); Conidiophore and conidia of *U. oudemansii* (3) (scales : 1, 3=10  $\mu$ m ; 2=5  $\mu$ m).

Fig. 4. *Verticillium lecanii*. Conidiophores and spore heads (scale : 4=10  $\mu$ m).

Figs. 5—9. *Verticillium* sp. Habit (5), conidia (6, 7) and conidiophores (6—8) near apex (7) and base (8), and chlamydospores (9) (scales : 5, 6=20  $\mu$ m ; 7—9=5  $\mu$ m).

Fig. 10. Unidentified,  $X_1$ . Sclerotium-like structure (scale : 10=10  $\mu$ m).

## クロマツとアカマツ種子から分離された 糸状菌と分類学上の知見

渡辺 恒雄<sup>(1)</sup>・植松 清次<sup>(2)</sup>・佐藤 幸生<sup>(3)</sup>

### 摘 要

クロマツとアカマツ種子を汚染している糸状菌についての知見は、我が国はもとより諸外国でも比較的乏しい。そこで長野県など6産地のクロマツおよびアカマツ種子を供試し、糸状菌を分離し、菌相を調べた。菌の分離は、できるだけ多くの種を分離する目的で、十分に水洗しただけの無消毒種子を用いた。すなわち各試料当たり300～500粒（合計2,000粒）の種子を水洗後素寒天培地上に置床し、25℃と10℃に2～10日間放置後、寒天培地上に伸び出た単菌糸を寒天とともに切り取って純粋分離株を得た。その結果、各試料当たり149～491菌株、合計1,615菌株を分離した。これらは未同定菌を除き41属（ただし、担子菌2菌株は便宜上1属として取り扱った）、53種、3グループ、33タイプに同定できた。その内訳は、接合菌と子のう菌各2属、担子菌2菌株と不完全菌36属であった。産地や分離温度などが異なると分離菌にも多少の違いが見られた。しかし、*Alternaria*, *Arthrinium*, *Aspergillus*, *Aureobasidium*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Penicillium*, *Pestalotia*, *Phialophora*, *Phoma*, *Trichoderma*, *Tripaspermum* の14属は、これら両種子のいずれかまたは両方で、一般的に見出された。また試料によっては、*Diplodia*, *Phomopsis* や未同定の  $X_1$  菌が高頻度で分離された。分離菌の中で、*Camposporium*, *Humicola*, *Pyrenochaeta*, *Sporidesmium*, *Verticillium* の各菌株は、既知のいずれの種にも該当せず新種の可能性が高いが、今後の検討課題としたい。さらに *Pestalotia* と *Phomopsis* 各2菌のマツ子苗への接種試験を行ったところ、各1菌株に病原性が認められ、分離菌の中に多数の種子伝染性植物病原菌が含まれていることが示唆された。

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(1) 保護部

(2) 千葉県暖地園芸試験場

(3) 富山県立技術短期大学

