Fungi Associated with Natural Regeneration of *Picea jezoensis* CARR. in Seed Stage

—Their distribution on forest floors and pathogenicity to the seeds—

By

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エゾマツ天然更新の種子段階に関連する糸状菌

—林床での分布及び種子に対する病原性を中心に—

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Abstract

Damage caused by fungi to the natural regeneration of *Picea jezoensis* CARR. in seed stage was investigated, by making fungal isolations from seeds which had overwintered in various forest floors and examining the pathogenicity of the isolated fungi and their distribution in forest floors in relation to seed germination. From the seeds, four fungi were frequently isolated: *Racodium therryanum* THUEM., *Arthrinium* sp., and two unidentified ones. Among these the most frequently isolated was *R. therryanum*, a well known pathogen causing dark snow blight to conifer seedlings in nursery. A highly significant negative correlation was found between the *R. therryanum* isolation percentages from the seeds and the germination percentages of the seeds, but no such correlations were found with the other fungi: the pathogenicity of *R. therryanum* to *P. jezoensis* seeds was demonstrated while that of the other fungi was not. The isolation of *R. therryanum* in relation to the forest floor types suggested that this fungus subsists neither on rotten fallen trees nor in soil-raked floors, but inhabits *Sasa*-growing or canopy-shaded floors and is distributed in such areas mainly in the A_0 layer rather than in the A layer of the soil. Removing the A_0 layer of the soil and sterilizing the seeds with TMTD both greatly lessened the seed decay caused by *R. therryanum*.

Key words: Seed decay, *Racodium therryanum* THUEM., natural regeneration, *Picea jezoensis* CARR.

1. Introduction

It is well known that natural regeneration of *Picea jezoensis* CARR. and *Abies sachalinensis* MAST., two important forest tree species in Hokkaido, does not usually

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occur except in only a few exceptional areas where the forest floors normally possess very particular site conditions. Numerous studies to identify the causes of this phenomenon have been made: usually involved with environmental factors such as soil, light, humidity, vegetation, etc. However, there have been few studies directed towards the fungal damage to the natural regeneration of such conifers though it has been suggested that fungal damage should not be neglected in considering forest regeneration.[8,9,11]

The first practical investigation on fungal damage to the natural regeneration of conifers in Hokkaido was made by HAYASHI and ENDO.[3] While participating in joint research to establish "management techniques of natural forest of *P. jezoensis* and *A. sachalinensis*", they found that one of the most important factors obstructing the regeneration of *A. sachalinensis* during the early growth stage was the seed decay in soil caused by fungus *Racodium therryanum* THUEM. However, since HAYASHI and ENDO's report of this finding many years has elapsed with no similar investigations made concerning *P. jezoensis*, a species considered more susceptible to fungal diseases than *A. sachalinensis*.[14] TAKAHASHI,[16] although he listed in his report some fungal diseases concerned in the natural regeneration of *P. jezoensis*, presented only a rough line with no details.

The objective of the present study is to obtain detailed information on fungal damage to the natural regeneration of *P. jezoensis* during seed stage. Though the study is still in progress, the authors have already reached some conclusions and herein wish to report them.

2. Study Site and Method

2.1 Study site

The study site is located in northern Hokkaido in Compartment 415 of the Uryu Experiment Forest of Hokkaido University (the experiment forest is situated in latitude 44°3′~29′N, longitude 142°1′~20′E, and altitude 200~700 m). The climate is as follows: mean annual temperature 3.5°C, with maximum and minimum temperatures reported in the last 10 years of 34.2°C in July and −41.2°C in Feb., respectively, showing a remarkably great difference between the two temperature extremes; indexes of warmth and coldness 46.7 and −66.0, respectively; annual precipitation 1,410 mm of which snowfall constitutes a large portion since the snow season lasts at least six months from the end of Oct. to the middle of May, with the maximum depth of accumulated snow reaching 2.75 m on flat ground.

Phytogeographically the forests here come into the zone of pan mixed forest of coniferous and broad-leaved trees, with the main tree species consisting of *A. sachalinensis*, *Picea glehnii* MAST., *Quercus mongolica* var. *grosseserrata* REHD. et WILS., and *Betula ermanii* CHAM. And *P. jezoensis*, though few, is also visible in some places. However, the forests on the whole are sparce and dotted everywhere with unstocked lands covered by *Sasa* (*Sasa senanensis*). With regard to Compartment 415 (altitude 350 m) in which the experiment plots were set, the forest is composed mainly of *A. sachalinensis* and *P. jezoensis*, with a few *Q. mongolica*

var. *grosseserrata*, *B. ermanii*, etc. mixed in. The soil belongs to brown forest soil and most of the floors are covered by *Sasa*. Further, as a means of assisting the natural regeneration of the forest, in some *Sasa*-covered unstocked lands, *Sasa* and surface soil were removed with raker-equipped bulldozer in 1982.

In the compartment described above, four typical kinds of floors, i. e., *Sasa*-growing ground, canopy-shaded ground, rotten fallen tree, and surface soil-raked ground, were selected for setting the experiment plots (seed beds). Descriptions of the four kinds of the floors are given below.

Sasa-growing ground : floor plants consisting of *Sasa* 1～1.5 m in height growing as densely as 70～80 culms/m², with a few other scattered herbs ; soil profile showing A_0 layer 6 cm thick, A layer 10 cm thick, and B layer ; further, the L layer of the A_0 layer 3～4 cm thick composed of defoliated leaves mainly of *Sasa* with a little from trees.

Canopy-shaded ground : no floor plants ; soil profile showing A_0 layer 8 cm thick, A layer 14 cm thick, and B layer ; further, the L layer of the A_0 layer 3～4 cm thick composed of defoliated needles of *A. sachalinensis* and *P. jezoensis*.

Rotten fallen tree : brown-decayed logs of fallen trees of *A. sachalinensis*, breast height diameter about 40～60 cm, lower part of the logs submerged in ground with upper surfaces for the most part covered by defoliated needles of *A. sachalinensis* and *P. jezoensis* with scattered moss also visible ; the decay having reached so severe a stage that the wood can be easily crushed by a push with fingers ; further, several large trees of *A. sachalinensis* and *P. jezoensis* probably aged over 100 years growing on the logs.

Surface soil-removed ground : unstocked ground with the B layer of the soil exposed due to removal of *Sasa* and surface soil by raker-equipped bulldozer ; no floor plants except for a few 2- or 3-year-old seedlings of *A. sachalinensis*, *B. ermanii*, etc.

The seed beds set in these four kinds of floors shall be called "*Sasa*-growing", "canopy-shaded", "fallen tree", and "soil-raked", respectively, in the remainder of the paper.

2. 2 Method

To three kinds of seed beds, i. e., "*Sasa*-growing", "canopy-shaded", and "fallen tree", three kinds of treatments were given, *Sasa*-cutting and A_0 layer-removing, A_0 layer-removing, and defoliated leaves-removing, respectively. An untreated control of each was also provided. In addition, these three treatments shall be generally called "A_0 removal" henceforth for convenience's sake. The seed bed of "soil-raked" remained untreated. All treatments as stated above were triplicated. As shown in Table 1, a total of 42 plots (each size being 0.3 m × 0.3 m) were set, of which 21 were prepared for untreated seeds, and the other 21 for seeds sterilized with fungicide. The seed sterilization was conducted by dressing the seeds with a hydrophilic powder fungicide TMTD (tetramethylthiuram disulfide content : 80%), with weight ratio of seed : TMTD being 100 : 3 (this fungicide is commonly used in nursery to control seedling dark snow blight and seed decay or seedling damping-off).

Table 1. Number of experiment plots for each treatment in every seed bed

Seed bed	No treatment	No treatment+ Seed sterilization	A_0 removal	A_0 removal+Seed sterilization	Total
Sasa-growing	3	3	3	3	12
Canopy-shaded	3	3	3	3	12
Fallen tree	3	3	3*	3*	12
Soil-raked	3	3	—	—	6
Total	12	12	9	9	42

* The removal of defoliated-leaves layer covering fallen trees is also called"A_0 removal" for convenience's sake.

On Oct. 23, 1984, just prior to the beginning of snow accumulation, into each of the plots were placed 100 seeds pocketed in a 10 cm-square-sized mesh bag made of nylon-cloth as a bait to trap the pathogenetical fungi. On May 2, 1985, immediately after the snow thawed, the seeds pocketed in the mesh bags were retrieved. A part of the retrieved seeds were used to isolate the fungi; the remaining seeds, to conduct the germination test. It should be noted that of the 100 seeds placed in each mesh bag not all were retrieved: due to the probable foraging by wild rats and other animals.

The isolation of fungi was carried out in the following manner: seeds were surface-sterilized for 5 min in 20% sodium hypochlorite solution; placed on potato sugar agar plates with a few drops of 10% phosphoric acid added, 10 seeds or less each plate; kept at 15～20°C for 4～20 days, and examined during this period for fungi. The germination test was conducted as follows: seeds were placed in porous clay dishes and kept at 20°C for 3 wks. during which the germinated seeds were counted and removed each day. (The germinated seeds were those which had developed radicles over 1 mm long.)

Then, with the fungi which were frequently isolated from the seeds, an artificial infection test was conducted to identify their pathogenicity. The fungi were initially incubated in wheat bran at 20°C for two months to prepare inocula; soil (commercially available for greenhouse use) was put into 10 cm diameter plastic pots with perforated bottoms and sterilized by autoclaving for 1 h at 15 lb pressure; 5 g of the inoculum prepared as described above was put into the autoclaved soil in each pot and mixed with the soil; 200 seeds, after being rinsed in running tap-water for 48 h, were sown in the inoculum-mixed soil in each pot and kept at 4～8°C in darkness for four months: three replicates were made for each fungus with controls also prepared in the same way but without the addition of the fungus inoculum. Subsequently, the seeds were taken from the soil and the fungal isolation and seed germination tests were conducted using the same procedures as described earlier. Furthermore, the infection test as stated above was also carried out using the TMTD sterilized seeds to confirm the effect of seed sterilization by TMTD on seed decay.

3. Results and Discussion

3.1 Taxonomy of fungi isolated from seeds

The fungi isolated from the seeds were categorized into 12 genera and 28 unidentified groups (Table 2). Of the identified fungi, only a few which were *Rhizopus* and *Chaetomium* belonged to *Phycomycetes* and *Ascomycetes*; the great majority were imperfecti fungi. This tendency agrees with the results of many investigations on forest soil fungal flora, for example, those reported by IGARASHI and MIZOGUCHI,[4] and ISHII.[5] Since these isolates from the seeds most likely had been soil-inhabiting fungi and thus might be a part of forest soil fungal flora, it may be only natural for a similar tendency to be exhibited in the isolation. However, it should be noted that the fungus *Racodium therryanum*, a well known pathogen causing dark snow blight to conifer seedlings in nursery,[6,7,10,14,17] was also isolated. Moreover, as already stated in the introduction, HAYASHI and ENDO[3] reported that this fungus plays an important role in obstructing the regeneration of *A. sachalinensis* by decaying the seeds in forest soils before the occurrence of germination.

Table 2. Taxonomy of fungi isolated from *Picea jezoensis* seeds

Family	Genus
Phycomycetes	*Rhizopus*
Ascomycetes	*Chaetomium*
imperfecti fungi	*Arthrinium, Cladosporium, Cylindrocarpon, Humicola, Papulospora, Penicillium, Racodium, Ramichloridum, Rinocladiella, Tritirachium*
unidentified fungi	W. C. (15 groups), H. C. (3 groups), Rh. C. (2 groups), others (8 groups)

Some fungi were identified to species level but only the genus names of them are given in the table.
For unidentified fungi: W. C.=white colony, H. C.=hyaline colony, Rh. C.=rhizomorphous colony, others=gray-green, gray-black, or drab colonies.

3.2 Fungal isolation related to types and surface-treatments of forest floors

Table 3 shows the number of fungus-isolated seeds from every seed bed for each isolated fungus. Much more fungi, both quantitatively and taxonomically, were isolated on the seeds from "*Sasa*-growing" and "canopy-shaded" seed beds than on those from "fallen tree" and "soil-raked" ones. Among all the fungi isolated, *R. therryanum* was the most numerously isolated, with the total number for the four types of the seed beds being 84: about 3 times that of W. C. 3, 6 times that of W. C. 1, and 7 times that of *Arthrinium* sp.[1,18] However, despite its isolation in great number, *R. therryanum* was isolated only on the seeds from "*Sasa*-growing" and "canopy-shaded" seed beds with none on the seeds from "fallen tree" and "soil-raked" ones. To some extent, W. C. 3 was evenly obtained on the seeds from all types of seed beds, while W. C. 1 was mainly from the "soil-raked" one. However, in contrast to W. C. 1, *Arthrinium* sp. was not isolated on the seeds from

Table 3. Number of fungus-isolated seeds from each seed bed for each isolated fungus

Isolated fungi	*Sasa*-growing			Canopy-shaded			Fallen tree			Soil-raked			Total
	I	II	III	I	II	III	I	II	III	I	II	III	
Racodium	10	16	19	6	20	13	0	0	0	0	0	0	84
Arthrinium	1	1	0	0	5	2	1	2	0	0	0	0	12
Ramichloridum	0	2	0	0	3	0	0	0	0	0	0	0	5
Tritirachium	1	1	0	0	1	2	0	0	0	0	0	0	5
Chaetomium	0	0	0	0	1	1	0	1	1	0	0	0	4
Rhinocladiella	0	0	0	0	0	0	2	2	0	0	0	0	4
Papulospora	1	2	0	0	0	0	0	0	0	0	0	0	3
Penicillium	0	0	0	1	1	0	0	0	0	0	0	0	2
Thielaviopsis	0	0	0	2	0	0	0	0	0	0	0	0	2
Rhizopus	1	0	0	1	0	0	0	0	0	0	0	0	2
Cladosporium	0	0	0	0	0	0	0	1	0	0	0	0	1
Cylindrocarpon	0	0	0	0	0	0	0	1	0	0	0	0	1
Humicola	1	0	0	0	0	0	0	0	0	0	0	0	1
unidentified:													
W. C. 3	3	11	1	2	2	0	1	2	1	0	1	2	26
W. C. 1	0	0	0	1	0	0	0	0	0	6	7	0	14
W. C. 5	0	5	0	0	1	1	0	0	0	0	0	0	7
W. C. 2	1	1	1	1	0	0	0	0	0	1	0	0	5
omitted	10	8	0	7	11	5	7	6	0	3	9	0	66

the "soil-raked" seed bed but was from the other three. With regard to the remainder of the isolates, a discussion will not be presented in this paper as their involvement with seed damage seems very unlikely due to the rarity of isolation.

Table 4 shows the isolation percentages of *R. therryanum* for each treatment in "*Sasa*-growing" and "canopy-shaded" seed beds. High isolation percentages ranging from 37% to 63% were obtained in the "no treatment" plots, while in "A_0 removal" plots, the isolation was found from one plot only with an isolation

Table 4. Isolation percentages of *Racodium therryanum* from *Picea jezoensis* seeds for each treatment in "*Sasa*-growing" and "canopy-shaded" seed beds

Seed bed	No treatment			No treatment +Seed sterilization			A_0 removal			A_0 removal +Seed sterilization		
	I	II	III	I	II	III	I	II	III	I	II	III
Sasa-growing	48	37	50	0	10	10	0	0	3	0	5	—
Canopy-shaded	0	63	43	0	0	0	0	0	0	29	5	0

Isolation percentages are calculated as follows: (number of fungus-isolated seeds/number of tested seeds)×100.
The mark "—" means missing sample.

percentage no more than 3%. A comparatively high isolation percentage reaching 29% found in one plot of "A_0 removal+seed sterilization" seemed very exceptional. However, this does not disprove the fact that the isolation percentages of *R. therryanum* in the "no treatment" plots were conspicuously much higher than those in the A_0 layer removed plots. The data concerning seed sterilization will be discussed later.

The data for W. C. 3 are shown in Table 5. The difference between the isolation percentages of W. C. 3 in "no treatment" plots and those in "A_0 removal" plots was not great, though the latter on the whole seemed a little higher than the former.

Table 5. Isolation percentages of unidentified fungus W. C. 3 from *Picea jezoensis* seeds for each treatment in every seed bed

Seed bed	No treatment			No treatment +Seed sterilization			A_0 removal			A_0 removal +Seed sterilization		
	I	II	III	I	II	III	I	II	III	I	II	III
Sasa-growing	5	7	0	0	3	3	9	7	0	6	13	—
Canopy-shaded	0	3	0	0	0	0	22	8	0	0	0	0
Fallen tree	0	5	0	0	0	0	14	4	6	0	0	—
Soil-raked	0	0	10	—	3	0	—	—	—	—	—	—

See "note" in Table 4.

The above results suggest that *R. therryanum* subsists neither on rotten fallen trees (sites of great particularity), nor in "soil-raked" grounds (sites which are deficient in organic matter), but inhabits the "*Sasa*-growing" or "canopy-shaded" grounds (sites which abound with organic matter) where its distribution is mainly in the A_0 layer rather than in the A layer of the soil. Also it is suggested that W. C. 3 may be a unbiquitous fungus in forest floors. *Sasa*-growing lands are estimated to account for 89% of all the forest lands in Hokkaido,[16] whereas the area occupied by fallen trees and "soil-raked" grounds or other mineral soil-exposed grounds are so small as not to be worth mentioning. In view of these facts, *R. therryanum* may have a very wide distribution range in forest lands of Hokkaido. As regards the distribution of *R. therryanum* in forest lands, no investigations have yet been made except that of ENDO, SANADA and KISHIDA[2] which reported that more *R. therryanum* were isolated on the *A. sachalinensis* seeds recovered from the A_0 layer than those from the A layer of the soil under the canopies of some broad leaved trees. The results of the present study support that of ENDO et al., and in consideration of the importance of *R. therryanum* as a pathogen to many conifer seedlings, may provide a stimulus to further and more detailed investigations on the distribution of *R. therryanum* in the forest lands in Hokkaido.

An interesting question to be covered in later studies is why *R. therryanum* was not isolated at all on the seeds from "fallen tree" seed beds, though these were also covered by defoliated needles as were "canopy-shaded" seed beds on the

seeds from which *R. therryanum* was numerously obtained.

3.3 The pathogenicity of the isolated fungi to seeds

The isolates obtained from the seeds were too numerous to test them all for pathogenicity to the seeds; therefore artificial infection tests were made only with the four dominant fungi. The results of the tests are shown in Table 6. The seeds taken from the soil inoculated with *R. therryanum* did not germinate at all: *R. therryanum* was reisolated from 99% of these seeds. On the other hand, none of the other three fungi, *Arthrinium* sp., W. C. 1, and W. C. 3, was reisolated from the seeds taken from the soils previously inoculated with them; also there were no evident differences between the germination percentages of these seeds and those of the control. The pathogenicity of *R. therryanum* to *A. sachalinensis* seeds was already shown by SATO,[13] HAYASHI and ENDO,[3] but that to *P. jezoensis* seeds had not been previously studied. The results of the present study demonstrated the pathogenicity of *R. therryanum* to *P. jezoensis* seeds; furthermore, it was also proved that *Arthrinium* sp., W. C. 1, and W. C. 3 were not pathogenetic to *P. jezoensis* seeds.

Table 6. Fungus isolation percentages and germination percentages of *Picea jezoensis* seeds taken from fungus-inoculated soils for each inoculum fungus

Inoculum fungus	Isolation test			Germination test		
	No. of seeds tested	Fungus isolation percentage		No. of seeds tested	Germination percentage	
		Mean	S. d.		Mean	S. d.
Racodium	50×3	99	1	100×3	0	0
Arthrinium	50×3	0	0	100×3	83	2.6
W. C. 1	50×3	0	0	100×3	76	3.1
W. C. 3	50×3	0	0	100×3	81	2.2
Control	50×3	0	0	100×3	78	2.1

S. d. = standard deviation.

3.4 Occurrence of seed decay in forest floors and associated fungi

Table 7 gives the germination percentages, for each treatment, of the seeds retrieved from forest floors. The seeds from the "no treatment" plots of the "*Sasa*-growing" floor gave a conspicuously low germination percentage (38%) compared with the seeds from the other plots (where germination percentages ranging from 63% to 88%): a decrease in germinating ability of *P. jezoensis* seeds occurred after the seeds had overwintered in some areas of the forest floor.

The relationship between the germination percentages and the fungus isolation percentages of the retrieved seeds from every plot (for *R. therryanum* and W. C. 3 only) is shown in Fig. 1. A highly significant negative correlation ($r = -0.91$; $P = 0.1\%$) was found between these two percentages for *R. therryanum*. The fungus

Table 7. Germination percentages of *Picea jezoensis* seeds retrieved from seed beds set in forest floors

Seed bed	No treatment	No treatment+ Seed sterilization	A_0 removal	A_0 removal+ Seed sterilization
Sasa-growing	38	75	88	63
Canopy-shaded	—	75	—	85

The germination percentage is the mean of three replicates; the mark "—" means missing sample.
The germination test for the seeds from "fallen tree" and "soil-raked" seed beds was not done due to the deficiency of the number of the retrieved seeds.

W. C. 3, though already proved not to be pathogenetic to *P. jezoensis* seeds, is also shown in Fig. 1 in comparison with *R. therryanum*. It is clear that the isolation percentage of W. C. 3 bears no relation to the germination percentage of *P. jezoensis* seeds. This result, along with that from the pathogenicity test discussed in section **3. 3,** indicates that the decrease in germinating ability of the overwintered *P. jezoensis* seeds shown in Table 7 was caused by *R. therryanum*, and that the possibility of W. C. 3 causing seed decay can be excluded.

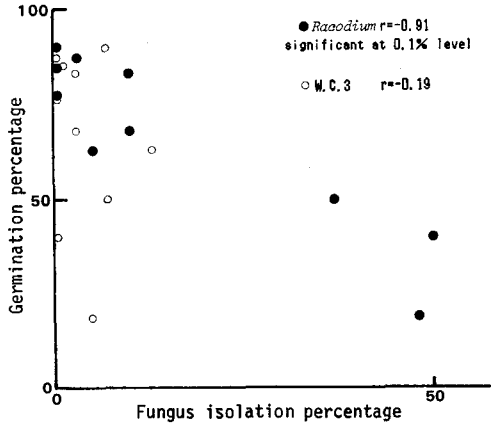

Fig. 1. Relationship between germination percentages and fungus isolation percentages of *Picea jezoensis* seeds retrieved from every experiment plot.

3. 5 Effect of surface soil-removing and seed sterilization on seed decay

The result showing the isolation percentages (37～63%) of *R. therryanum* in "no treatment" plots to be conspicuously higher than those (0～29%) in "A_0 removal" plots (Table 4) was already discussed in section **3. 2**: this result indicates that removing the A_0 layer of the soil off the forest floor will greatly reduce the amount of *R. therryanum* in the soil. Therefore, it is reasonable to consider A_0 layer removal as an effective means of preventing the seed decay caused by *R. therryanum*.

Also it can be seen from Table 4 that seed sterilization markedly inhibited *R. therryanum* from infecting the seeds in the case of "no treatment" plots. However, no inhibiting effect was perceptible in the case of "A_0 removal" plots: probably due to the scarcity of *R. therryanum* in such plots. The effect of seed sterilization on seed decay in the pathogenicity test is shown in Table 8: for unsterilized seeds, *R. therryanum* was reisolated from 99%, and none of these seeds germinated; however, for the sterilized seeds, *R. therryanum* was regained from only 23% and the germination percentage was kept at 51%. In view of the above results, seed

Table 8. Effect of seed sterilization by TMTD on seed decay caused by *Racodium therryanum*

Seed treatment	Isolation test			Germination test		
	No. of seeds tested	Fungus isolation percentage		No. of seeds tested	Germination percentage	
		Mean	S. d.		Mean	S. d.
sterilized	50×3	23	4.8	100×3	51	1.7
unsterilized	50×3	99	1	100×3	0	0

S. d. = standard deviation.

sterilization by TMTD may be deemed an effective method to prevent or lessen the seed decay caused by *R. therryanum*. SATO[12,13] reported that the fungicide TMTD gave good results in the prevention of *A. sachalinensis* seed decay. The authors' result about *P. jezoensis* seeds provided substantiating evidence to that of SATO.

4. Conclusions

Due to the seed decay caused by fungus *R. therryanum*, the *P. jezoensis* seeds which dropped into forest floors suffered losses of germinating ability. The fungus *R. therryanum* not only possesses a strong pathogenicity to *P. jezoensis* seeds, but is very likely widely distributed in forest lands except some particular sites such as rotten fallen trees or organic matter deficient areas (surface soil-removed lands). Accordingly, the seed decay caused by *R. therryanum* should be regarded as an important obstructive factor to the natural regeneration of *P. jezoensis* in the seed stage (from seed dropping into soil until seed germination).

Seed sterilization by TMTD is an effective means of preventing the seed decay caused by this fungus, but its application to aid the natural regeneration seems to be difficult. However, since this fungus mainly inhabits the A_0 layer of the soil, it can be concluded that its amount in the soil will be greatly reduced if the A_0 layer is removed; thus the potential germinating ability of the seeds which dropped into the soil will be maintained. As a means of assisting the natural regeneration of *P. jezoensis*, removal of the A_0 layer of the soil seems to be worth considering. Recently, removing *Sasa* and surface soil off the *Sasa*-growing forest floors by raker-equipped bulldozer has been becoming a popular forest procedure of aiding the natural regeneration of forests in Hokkaido: the results of the authors' study provide theoretical support for this forest procedure.

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要　　約

エゾマツ天然更新の種子段階における菌害を究明することを目的とし，天然林内の各種林床で越冬したエゾマツ種子から糸状菌の分離を行い，分離された菌について，その病原性，その林床での分布などを種子の発芽率と結びつけ更新に及ぼす影響について考察した。

種子から *Racodium therryanum*, *Arthrinium* sp., 及びその他の二種未同定糸状菌が高頻度で分離され，その中で分離頻度の最も高かったのは，暗色雪腐病原菌として知られている *R. therryanum* であった。*R. therryanum* の検出率と種子の発芽率との間に負の高い相関関係が示され，さらに接種試験によりこの菌はエゾマツ種子に対し強い病原性を持つことが確認された。その他の菌については，いずれも種子の発芽阻害とは関係ないことが判明した。また，*R. therryanym* は，天然林内において，倒木のような特殊な立地，またはかき起こし跡地のような腐植質に欠けたところには存在しないのに対し，ササ型林床，林冠下などのような腐植質に富んでいるところに分布し，かつ A_0 層における分布量が A 層より多いことが明らかになった。さらに，林床からの A_0 層除去とチウラム殺菌剤による種子消毒のいずれも，当菌による種子腐敗を大幅に軽減することが認められた。

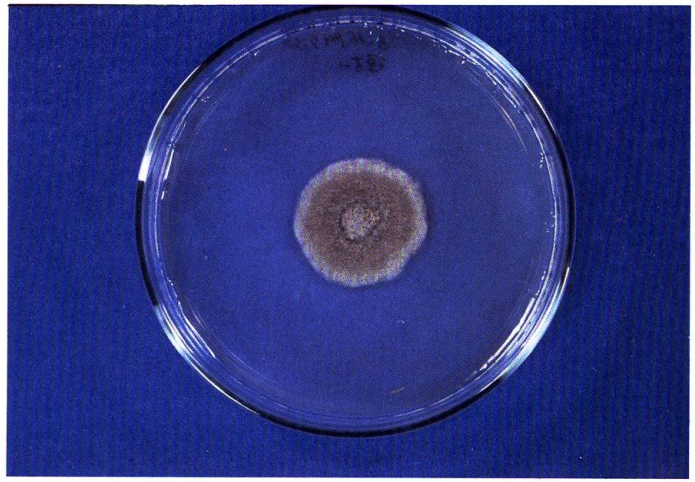

Photo 1. Colony of *Racodium therryanum* on PSA plate (20°C, 14 days)

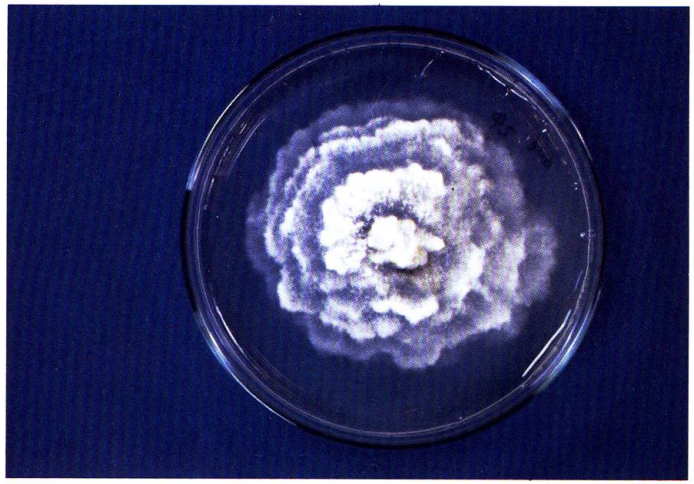

Photo 2. Colony of W.C.3 on PSA plate (20°C, 14 days)

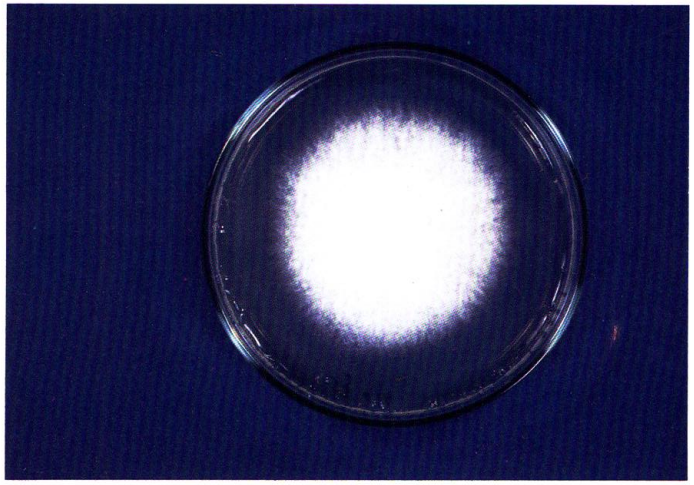

Photo 3. Colony of W.C.1 on PSA plate (20°C, 14 days)

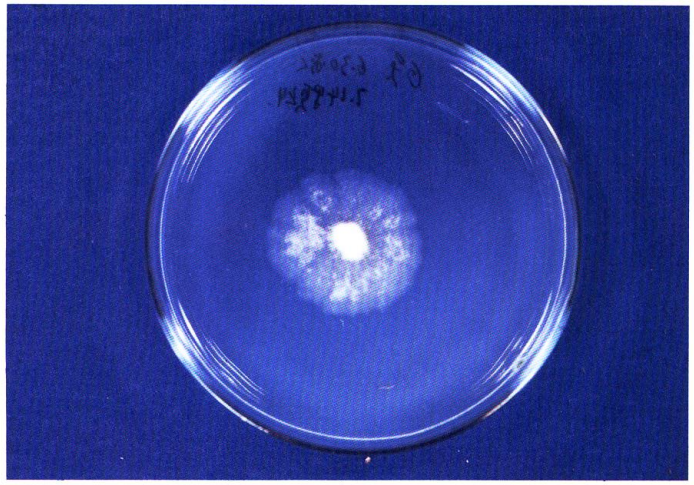

Photo 4. Colony of *Arthrinium* sp. on PSA plate (20°C, 5 days)

Photo 5. Mycelia of *Racodium therryanum* are growing from the decayed *Picea jezoensis* seed

Photo 6. Cross sections of *Picea jezoensis* seeds: decayed by *Racodium therryanum* (left); sound (right).

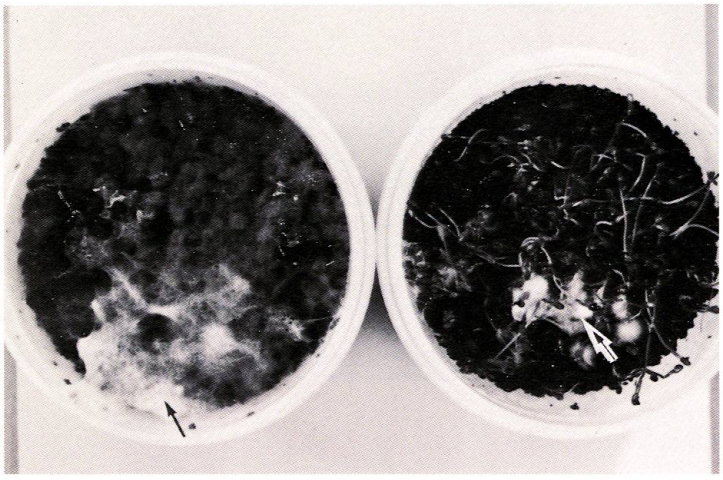

Photo 7. Germination of *Picea jezoensis* seeds in *Racodium*-inoculated soil (left) and that in control soil (right). White mycelia (arrows) are of a contaminator identified as *Fusarium* sp.