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Fungal Damage Caused by *Racodium therryanum* to Regeneration of Japanese Larch by Natural Seeding

By

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カラマツの天然下種更新を阻害する暗色雪腐病菌
(*Racodium therryanum*)

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Abstract

Occurrence of Japanese larch (*Larix kaempferi* CARR.) seed decay caused by *Racodium therryanum* THUEM., the causal fungus of dark snow blight, in forest soils of different layers was investigated both in laboratory and in field. Seed germination ability decreased severely in soils of surface organic layers (L, F-H) and less severely in soils of less organic layer (A), but not at all in the mineral soils of B layer. The high percentage of *R. therryanum* isolation from the seeds was found only in soil of surface organic layers, suggesting that the great majority of the *R. therryanum* population in forest soil inhabit the surface organic layers. The regeneration of Japanese larch by natural seeding is known to be restricted to the locations where the soils are deficient in organic matter. The occurrence of *R. therryanum*-caused Japanese larch seed decay, related to the organicity of the soils as shown in the present study, can in part explain such restricted regeneration pattern of Japanese larch.

Key words: Seed decay, *Racodium therryanum* THUEM.,
regeneration by natural seeding, Japanese larch.

1. Introduction

Japanese larch (*Larix kaempferi* CARR.) was introduced to Hokkaido in the late Meiji Period, and came to be planted extensively from the 1950s due to its quick growth. The area of the Japanese larch plantations has continually increased and has reached 490,000 ha. which makes up about 33% of the total forest plantation in Hokkaido⁵⁾. However, the early boom in the Japanese larch planting has slumped in recent years due to the depressed Japanese larch timber market, causing further problems in terms of forest resource conservation.

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In the mean while, regeneration by natural seeding of Japanese larch has been observed in locations around Hokkaido for a long time. Little attention has been paid to this phenomenon although attempts to reactivate the depressed Japanese larch forestry through use of such unattended natural regeneration appears promising. The possibilities of such an attempt have been suggested by the senior author and others in the previous report⁶⁾, in which the actual conditions of the natural regeneration of Japanese larch in Hokkaido was discussed.

As surveyed in this report, natural regeneration of Japanese larch is restricted to locations such as side-slopes of forest roads, dry river beds, etc. where the mineral soil is exposed due to the loss of surface organic soil for various reasons. However, such restricted regeneration pattern is also found in other forest tree species in Hokkaido, e. g., Yezo spruce (*Picea jezoensis* CARR.) and Sakhalin fir (*Abies sachalinensis* MAST.), etc.

Fungal damage caused by *Racodium therryanum* THUEM., the causal fungus of dark snow blight, can be reasonably considered as an important cause inducing such site restriction on regeneration of Yezo spruce and Sakhalin fir. It was reported that seeds of these two species overwintering in organic soils were severely decayed by *R. therryanum*^{1,4)}, but those in organic layer-removed soils were not¹⁾. However, though the seeds of Japanese larch were also found to be susceptible to *R. therryanum* infection³⁾, the extent and effects of the disease on the natural regeneration of Japanese larch are unknown.

We herein report the occurrence of Japanese larch seed decay caused by *R. therryanum* in forest soils of various types, with a discussion on the relation of this fungal damage to the regeneration of Japanese larch by natural seeding.

2. Materials and Methods

2. 1 Laboratory experiment

Soil sampling was conducted in a 32-year-old Japanese larch plantation situated near the Town of Okkope in northern Hokkaido. In this stand, Japanese larch seedlings generated in quantities by natural seeding occur in the stand gaps where surface organic layers (A₀-A) of the soil had been removed with raker-equipped bulldozer in 1982 or 1985 to promote the Japanese larch regeneration by natural seeding in these gaps. Two sets of soil samples were collected: one from Compartment 18 and the other from Compartment 19. Each set contained two samples, one of which was from the surface organic layer-removed ground where, as stated above, naturally regenerated Japanese larch seedlings occur in great number; the other was from the forest floor nearby where the organic layer of the soil was untouched. The former sample, hereafter called "B," consisted of B layer-soil sampled at a depth of about 5cm beneath the ground surface, and the latter, hereafter called "F-H," consisted of F-H layer-soil obtained by sampling the soil after removing the L layer.

Each of the soil samples was tripartitely divided as three replicates and put into three lidded and bottom-perforated pots. Each 100 seeds were pocketed in a nylon-cloth mesh bag then rinsed in running tap water for 48 hr., and buried in the soil in each pot. The seeds used were collected from a Japanese larch plantaion situated near the City of

Shibetsu, and possessed a germination percentage of 61%. The soils with pocketed seeds were incubated at 5°C in darkness for four months. Then the seeds were taken from the soils and the germination and fungus isolation tests were conducted on these seeds. Of 100 seeds in each bag, 50 were used for germination test, and the other 50 for fungus isolation test. The germination test was done by incubating the seeds on a quartz-stand supported moistened filter paper in Petri dish at 23°C for 4 wks then counting the germinated seeds. The fungus isolation test was performed using the procedure as described earlier¹⁾ and the fungi isolated were identified. The number of *R. therryanum*-isolated seeds in each replicate of 50 seeds was recorded and expressed as *R. therryanum* isolation percentage of each replicate. Finally the mean \pm SD of each three replicates for both germination percentage and *R. therryanum* isolation percentage was calculated, and correlation coefficients between the seed germination and the *R. therryanum* isolation were determined based on the data each of which represents one 50-seed replicate.

2. 2 Field experiment

The study site is located in a 35-year-old Japanese larch stand situated in Misumai in the suburbs of Sapporo. The soil in the stand belongs to brown forest soil and most of the floors are covered by *Sasa* bamboo (*Sasa senanensis*). On Oct. 30, 1986, just prior to the beginning of snow accumulation, three plots (seed beds) were set as three replicates after cutting away the *Sasa* clumps around. Each of the plots was 20cm square, and fenced up at four sides with four pieces of plastic boards 2mm thick and 15cm high, which were inserted 10cm-deep into ground in an attempt to prevent wild rats from entering from below. Each plot was further quartered into four quadrats by inserting three more pieces of plastic boards of different width. The four quadrats in each plot, after being given different treatments as shown in Table 1, were regarded as representative of different soil layers, i.e., L, F-H, A and A' layers, respectively (Table 1).

After this, each set of 100 seeds (as used in the previously-described laboratory experiment) pocketed in a mesh bag were placed on the soil in each quadrat. Then each plot was covered with a regularly cubical wire netting basket to prevent wild rats from disturbing the experiment. On May 10, 1987, soon after the snow thawed, the seeds were retrieved and the germination and fungus isolation tests were undertaken with the same procedures as described in section 1.

Table 1. Descriptions of the quadrats in each plot after different upper soil-removing treatments.

No. of Quadrat	Treatment	State of Quadrats after Treatment	Soil Layer Represented
1	No treatment	Covered by litters	L
2	Removing L layer only	F-H layer exposed	F-H
3	Removing L, F-H layer	A layer exposed	A
4	Removing L, F-H and upper A layer*	Internal soil of A layer exposed	A'**

* Upper soil of A layer was removed to a depth of about 2 cm.

** The term "A'" is not a standard call for soil layer, but only used here for convenience.

3. Results

3. 1 Seed decay in different soil samples

The results of the germination of Japanese larch seeds and *R. therryanum* isolation from these seeds which had been incubated in different sampled soils are shown in Table 2. The mean germination percentages of seeds incubated in F-H soils sampled from two compartments were only 7% and 13%, and the mean *R. therryanum* isolation percentages from these seeds reached 48% and 74%. However, for seeds incubated in B soils, the mean germination percentages were as high as 60% and 63% which do not differ much from the germination of the untreated seeds (61%), and no *R. therryanum* isolate was found.

Table 2. Results of germination of Japanese larch seeds and *Racodium therryanum* isolation from these seeds which had been incubated for 120 days at 5°C in soils of different types sampled from two compartments of a Japanese larch plantation in Okoppei

Soil Type	Germination %		Isolation %	
	Comp. 18	Comp. 19	Comp. 18	Comp. 19
F-H	7±4.1	13±9.8	48±28.0	74±15.2
B	60±3.4	63±6.5	0	0

Data are means±SD of three replicates of 50 seeds each.

The relationship between the germination and the *R. therryanum* isolation based on the data of 12 points, each of which represents the data of each replicate of 50 seeds, is shown in Fig. 1. It is obvious that the germination of the seeds after being incubated in different soils as negatively correlated to the *R. therryanum* isolation from these seeds ($r = -0.87$; $P = 1\%$).

3. 2 Naturally occurred seed decay on different soil layers in field

Table 3 shows the results of germination of Japanese larch seeds and *R. therryanum* isolation from these seeds which had overwintered on different soil layers in field. Seeds having overwintered on L, F-H and A layers gave conspicuously low mean germination percentages (13%–12%) as compared with those of the seeds on A' layers (63%), but possessed much higher mean *R. therryanum* isolation percentages (49%–71%) than the latter seeds.

Fig. 2 gives the relationship between the germination and *R. therryanum* isolation based on the data each of which represents one re-

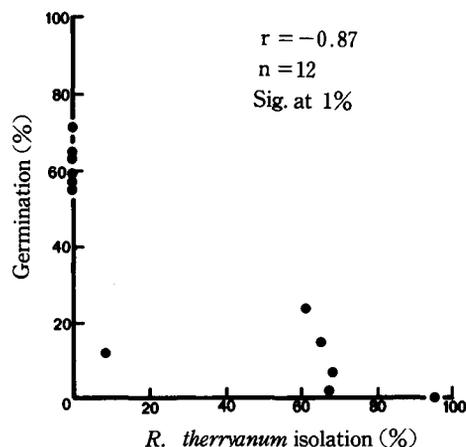


Fig. 1. Relationship between germination and *Racodium therryanum* isolation of Japanese larch seeds after being incubated in soils sampled from a Japanese larch plantation: each data point represents the data of one replicate of 50 seeds.

Table 3. Results of germination of Japanese larch seeds and *Racodium therryanum* isolation from these seeds which had overwintered on different layers of the soil in a Japanese larch plantation in Misumai.

Soil Layer	Germination %	Isolation %
L	19±16.5	65±40.1
F-H	21±23.0	49±36.2
A	13± 9.3	71±29.3
A'	63± 1.5	11± 5.5

Data for L, F-H and A are means±SD of three replicates of 50 seeds each; those for A' are of two replicates due to the missing of one sample.

For information on A' soil layer, see Table 1.

uplicate of 50 seeds. The negative correlation ($r = -0.89$) between the two was highly significant ($P = 1\%$).

4. Discussion

The results concerning seed germinations showed that loss of germination ability of Japanese larch seeds did occur to greater or less degree in forest soils, no matter whether under controlled condition or under field condition. The close negative correlations between the germination and the *R. therryanum* isolation (Fig. 1 and 2) suggested that the loss of germination ability of the seeds was due to the infection from *R. therryanum*, though, in fact, this can be considered as true beyond doubt in view of this fungus's strong pathogenicity to Japanese larch seeds, which has already been demonstrated³⁾.

Consequently, the quantitative diversity of seed germinations which appeared to be related to soil types or soil layers and is perceptible from Table 2 and 3, can be readily explained by the differences in the amount of *R. therryanum* inoculum in the different soils. The results of *R. therryanum* isolations showed that this explanation is reasonably acceptable. The mean *R. therryanum* isolations from the seeds which had been brought into contact with organic soils, i. e., L, F-H, and upper A layers, were conspicuously high (48%-74%), but those from the seeds having come into contact with less organic soils, i. e., the internal soil of A layer (called A' for convenience), was obviously low (11%), and the *R. therryanum* isolations became still lower (reduced to 0) in the case of mineral soils, i. e., the B layer. These facts suggested that the largest amount of *R. therryanum* inoculum are distributed in the surface organic layers (L and F-H layers) of the soil, and with the depth increasing further from the surface of A layer, the population level of *R. therryanum* begins to drop sharply and reduces to 0 in B layer. This suggestion is further supported by several previous studies of the authors¹⁾ and others²⁾ in which the similar trends in the relation between the *R. therryanum* isolation and the organicity of the soil

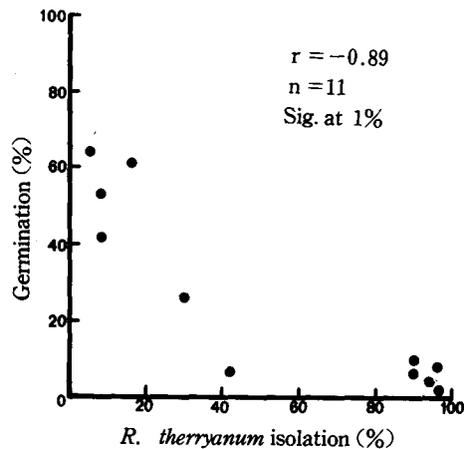


Fig. 2. Relationship between germination and *Racodium therryanum* isolation of Japanese larch seeds which have overwintered on soils of different layers in a Japanese larch plantation: each data point represents the data of one replicate of 50 seeds; a replicate is missing.

were found.

R. therryanum is well known to cause dark snow blight to coniferous seedlings at nurseries, but is less known for its role as a seed pathogen in forest regeneration in spite of its strong pathogenicity to seeds of many forest tree species as already demonstrated^{1,3,4,7)}. The regeneration of Yezo spruce and Sakhalin fir by natural seeding was found to be hindered by *R. therryanum* since this fungus induces germination failure of the seeds by causing seed decay in forest soils^{1,4)}. The present study showed for the first time that the regeneration of Japanese larch by natural seeding also suffers the same kind of damage caused by *R. therryanum*.

Moreover, the senior author and others have surveyed the actual conditions of the Japanese larch's natural regeneration in Hokkaido⁶⁾, and found that the regeneration of this forest tree is strictly restricted to the locations where the soils are deficient in organic matter. The distribution pattern of seed pathogen *R. therryanum* in the soils as shown from the present study's results can in part explain this phenomenon.

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- *2)-7) are originally in Japanese with some possessing English summaries.

要 約

人工林のカラマツを母樹とする天然更新は、北海道の各地で見ることができる。しかし、更新の立地は、道路法面・集材路・かき起し地などの有機質に富む土壌が除去され鉱物質土壌が露出した場所に限られる。有機質に富む土壌では、エゾマツなどの種子についての調査によ

ると、暗色雪腐病菌 (*Racodium therryanum*) による種子腐敗がよく発生し、この菌の被害が更新を阻害する要因の一つとなっている。そこで、カラマツの更新もエゾマツと同様にこの菌の侵害により阻害されていることを予測して、確認のための実験を行った。

有機質に富む F-H 層及び鉍物質土壌の B 層から土壌サンプルを採取し、これらの土壌サンプルにカラマツ種子を一定期間埋土した後、種子の発芽率と菌の侵害率を調査した。その結果、種子の発芽率の低下は、F-H 層土壌で激しく、B 層土壌では認められなかった。菌の侵害率は、F-H 層土壌に埋土した種子で非常に高く、B 層土壌に埋土した種子ではゼロであった。さらに、野外において各土壌層位で越冬させたカラマツの種子についても同様な調査を行った。その結果、L 層、F-H 層の土壌では、種子の発芽率の低下は著しく、菌の侵害率も上部土壌を除去した A 層より顕著に高かった。

以上の結果から、カラマツの天然更新においてもエゾマツと同様に暗色雪腐病菌によって阻害されることが明かとなった。また、高い菌の侵害率が常に L、F-H 層の土壌で発生することから、暗色雪腐病菌が主に林床の有機物に富む表層に生息することが示唆された。有機質に富む土壌にカラマツの下種更新が認められないのは、落下種子が暗色雪腐病菌の侵害を受けるためであり、鉍物質土壌には、重要な阻害要因である本菌が存在しないために下種更新が行われると言えよう。