

Effects of Stratification on the Germination, Respiration and Water Uptake of *Pinus koraiensis* Seed.

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Stratification, i. e. prechilling treatment of seed under moist condition, is often favored in nursery practice of forestry, and it is also repeated in natural stand during winter.

The causes of its effect, however, are not yet elucidated enough. Physical degeneration of seed coats is possibly estimated, but the conclusive determination seems to be not obtained. Other evidences have been shown with regard to the change of physiological status of resting cells in seed during its after-ripening, that is, the chemical change of reserve substances, the activation of some enzymes, the elevation of growth promoter and others (cf. HATANO and ASAKAWA, 1964).

POLLOCK and OLNEY (1959, 1960) have reported the stimulated respiration with after-ripening of cherry seed and its relation to phosphate metabolism. More recently, the finding by NYMAN (1963) with its effect on pine seed, substituted for photo-reaction, emphasizes the necessity of further study on the metabolic change with stratification.

The germination of *Pinus koraiensis* seed is accelerated by means of stratification (TOZAWA, 1926; BARTON, 1930; ASAKAWA, 1955). The seed is seemingly adequate as the object for such analytical study because of one of the largest seeds in Genus *Pinus*, exclusive of covering by the thick and compact testa (UEKI, 1927).

Therefore, this study aimed to clarify the participation of respiratory relations in stratification with the pine seed.

Material and Methods

Seeds of *Pinus koraiensis* SIEB. et ZUCC. were supplied generously by Dr. HYUN from Korea at the first of 1963 and by the members of Tokyo University Forest from Hokkaido in the fall of 1963. The seeds were reserved in glass bottles at 0-5°C up to the experiment. The experiment was carried out between late summer of 1963 and early summer of 1964. The seed of Korea origin was used only for the germination test, and major parts of the experiment were assayed with that of Hokkaido origin.

Concerning the pretreatment of seed coats, the seed was punctured by pincers about the edge of testa, at which the rootlet should be protruded, and in the case of 'decoating' the testa and inner coat were removed completely (Fig. 1).

Before sowing seeds on germination bed with filter paper on moistened vermiculite in PETRI dish (50 seeds in each dish), disinfected previously in KOCH's steamer, the intact seed was treated with 0.1 percent HgCl_2 for 30 minutes, the punctured seed for

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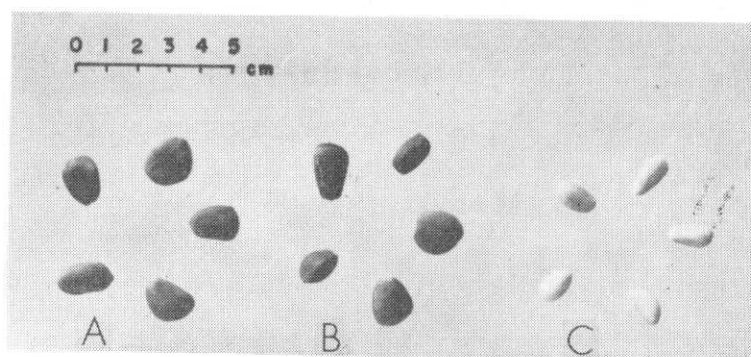


Fig. 1. *Pinus koraiensis* seeds, treated with the seed coats.
A: intact seeds; B: punctured seeds; C: decoated seeds.

15 minutes, and the decoated seed for 5 minutes, then rinsed with aseptic distilled water to remove microbial contamination.

Prechilling period at 0–5°C for these seeds, previously treated with seed coats or untreated, was limited to 30 days in all cases. The seeds, stratified or unstratified, were incubated in a thermostat for the germination at 25°C in darkness, but sometimes opened for seed-counting. The germination with intact and punctured seeds was judged by root-tip protrusion through testa, and so with the decoated one by its direct protrusion from endosperm. The values of germination test were obtained by adding two lots, accordingly on 100 seed basis.

Oxygen uptake was measured with the conventional WARBURG manometer under ordinary room light on seed materials at the initial germination stage at 25°C (HATANO, 1963). The interior of the cup was kept moistened on imbibed filter paper. The carbon dioxide produced was absorbed in 0.2 ml of 10 percent KOH with filter paper for spreading in the center well. CO₂ output was determined by the direct method using three flasks, the two with CO₂ absorbant and the other without it. No correction was made for CO₂ retention in the medium in the manometer. The manometer was oscillated 120 times per minute.

Before putting seeds or seed organs into the cup, the decoated seed was once more treated with 0.1 percent HgCl₂ for 5 minutes, and then rinsed with aseptic water. The operation to dissect endosperm and to isolate embryo, when necessary, was made immediately before each manometer test.

In the assay with 2,4-dinitrophenol (DNP), its aqueous solution (0.3 ml) was poured from the side chamber on un-moistened filter paper in the main chamber, through which the reagent should be absorbed into the embryo.

After the respiration measurement, the plant materials were assayed for its water content as the difference between fresh and dry weight estimated after being dried at 105°C for 12 hours.

The values of oxygen uptake and water content were obtained with duplicate or by single measurement.

Results

The effect of stratification on seed germination was compared among the intact, punctured, and decoated seeds. Fig. 2 shows that the treatment accelerated slightly germination speed in the intact and decoated seeds, although its effect was nullified in the punctured seed. On the other hand, decoating treatment was very effective for the germination of both seeds stratified and unstratified. But, the embryos in these seeds of all cases seem to scarcely grow during stratification (Table 1).

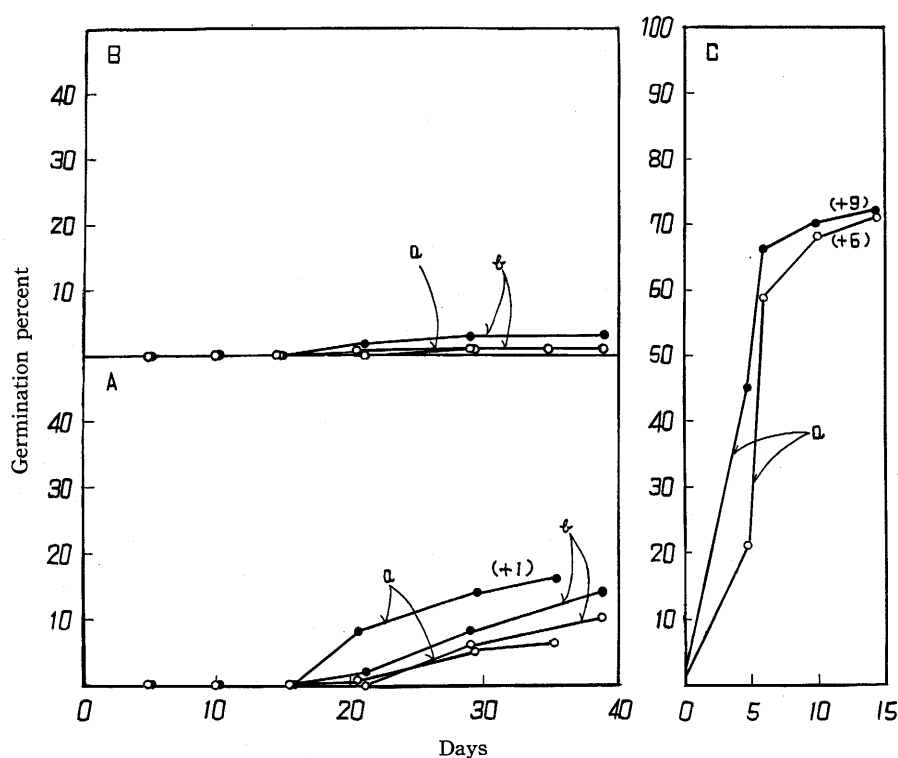


Fig. 2. Germination of the seeds.

A, B, and C: same in Fig. 1. a: Korea origin. b: Hokkaido origin. Filled circles: stratified for 30 days. Empty circles: unstratified. Germination percent indicates percentage on basis of seed number excluding empty seeds. Numbers in parentheses show abnormal germination percent. Abscissa: incubation period.

Table 1. Embryo ratios in the stratified and unstratified seeds of *Pinus koraiensis*.

	Stratified			Unstratified		
	intact	punctured	decoated	intact	punctured	decoated
A (before transfer to 25°C)	0.57	0.61	0.62	0.56		
B (after 4 day incubation at 25°C)	0.59	0.59	Some seeds germinated	0.54	0.57	Some seeds germinated

Embryo ratio was expressed as percentage of embryo length per endosperm.

A: average of 50 seeds; B: average of 25 seeds.

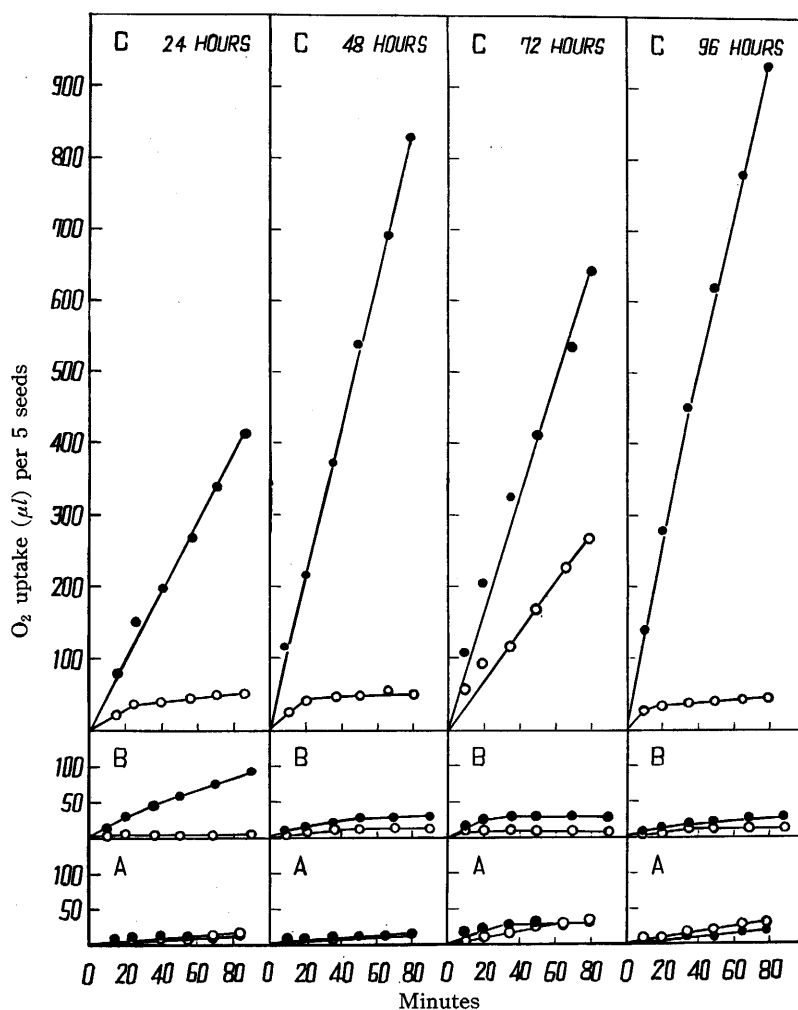


Fig. 3. O_2 uptake in the germinating seeds.

A, B, and C: same in Fig. 1. Filled and empty circles: same in Fig. 2. Hours in the figure indicate period of seed incubation at 25°C . The samples in A lots of 48 and 72 hours contained 2 empty seeds each. Abscissa: time of measurement.

Table 2. Change of O_2 uptake in the embryos isolated from decoated seeds and the dissected endosperms.

Incubation period of decoated seeds at 25°C (hours)	Unstratified				Stratified			
	Embryo		Endosperm		Embryo		Endosperm	
	A	B	A	B	A	B	A	B
0	7.5*	11.0	10.2*	19.9	20.1*	20.1*	87.5*	145.7
20	7.6	13.4	24.3	29.6	22.6	30.1	160.8	180.9
45	15.9	25.1	36.5	133.1	28.4	40.2	330.6†	160.2†
69	5.9	10.1	67.9	60.9	33.5	42.7	71.7	71.2
93	33.5	36.9	170.7	57.0	37.7	41.7	51.3	5.2

Values in the table show O_2 uptake (μl) of 5 seeds per 30 minutes.

A: between 1 and 2 hours after the operation to dissect and isolate;

B: between 5 and 6 hours after the operation.

(With asterisks) indicate the values between 3 and 4 hours, and (with daggers) were obtained by doubling of 15 minute values.

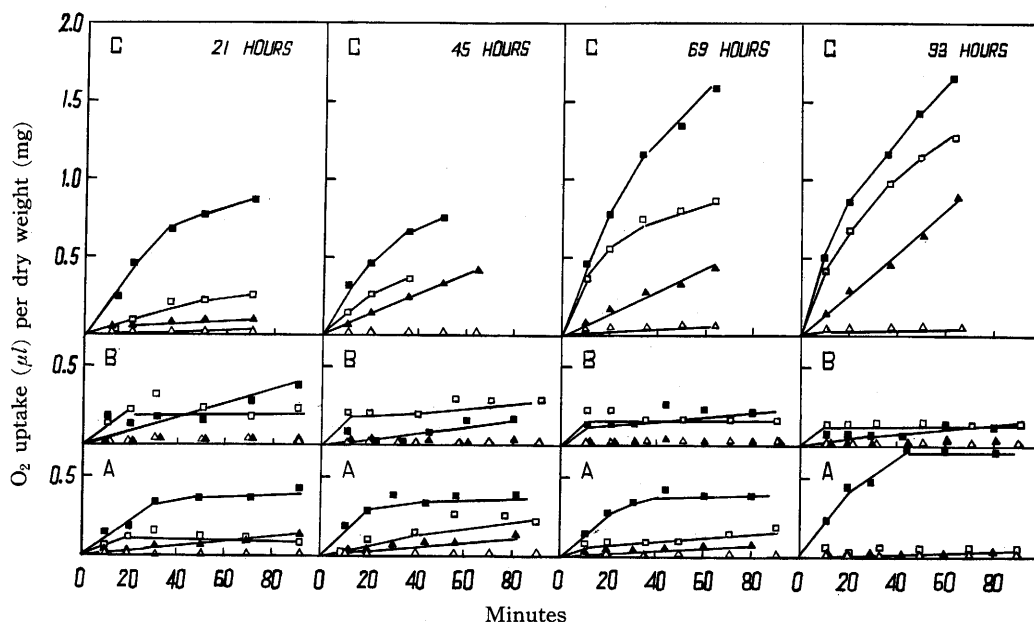


Fig. 4. O₂ uptake in the isolated embryos and the dissected endosperms. Filled squares: embryos isolated from stratified seeds; empty squares: embryos isolated from unstratified seeds. Filled triangles: endosperms dissected from stratified seeds; empty triangles: endosperms dissected from unstratified seeds. A, B, and C: same in Fig. 1. Hours in the figure indicate period of seed incubation at 25°C before the operation. The measurement was performed between 3 and 4.5 hours after the operation. Abscissa: same in Fig. 3.

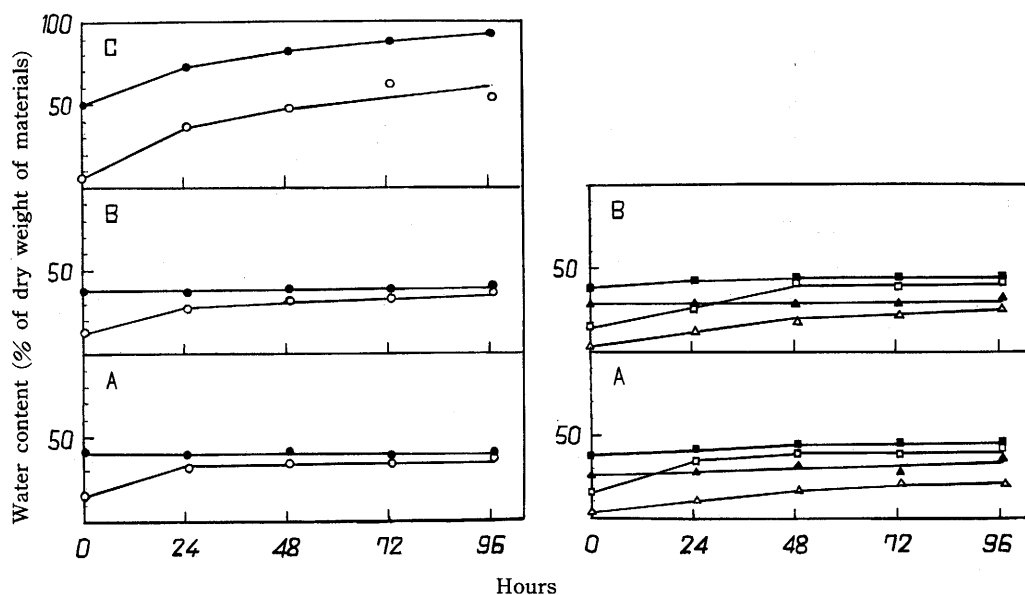


Fig. 5. Water uptake in the seeds. Left (filled circles: stratified seeds; empty circles: unstratified seeds). Right (filled triangles: embryos plus endosperms of stratified seeds; empty triangles: embryos plus endosperms of unstratified seeds. Filled squares: testas plus inner coats of stratified seeds; empty squares: testas plus inner coats of unstratified seeds). A, B and C: same in Fig. 1. Abscissa: incubation period.

At the initial stage of seed germination, oxygen uptake was enhanced in the decoated, stratified seed, as shown in Fig. 3. Table 2 shows the change of O_2 uptake in the dissected endosperm and isolated embryo from that seed. O_2 uptake in the embryos increased steadily with time elapsed after isolation, while that of endosperms

Table 3. Water content in the isolated embryos and dissected endosperms.

Incubation period of seeds at 25°C (hours)	Stratified						Unstratified					
	embryo			endosperm			embryo			endosperm		
	A	B	C	A	B	C	A	B	C	A	B	C
0	63.0	72.6	145.9	25.4	28.5	44.7	15.0	15.0	15.0	5.6	5.6	5.6
21	131.2	174.3	192.4	32.6	34.0	60.8	105.1	133.2	112.4	26.2	32.7	32.6
43	113.4	137.4	196.8	39.3	41.5	62.5	123.0	104.5	129.3	34.5	29.4	42.8
69	136.1	148.2	225.8	32.4	36.4	70.0	91.7	106.8	161.0	27.4	30.0	59.5
93	146.5	141.5	265.7	42.1	42.5	85.2	139.7	127.5	196.8	29.9	30.2	67.1

Values in the table: percentage of water content in dry weight of materials.

A, B and C: intact, punctured and decoated seeds.

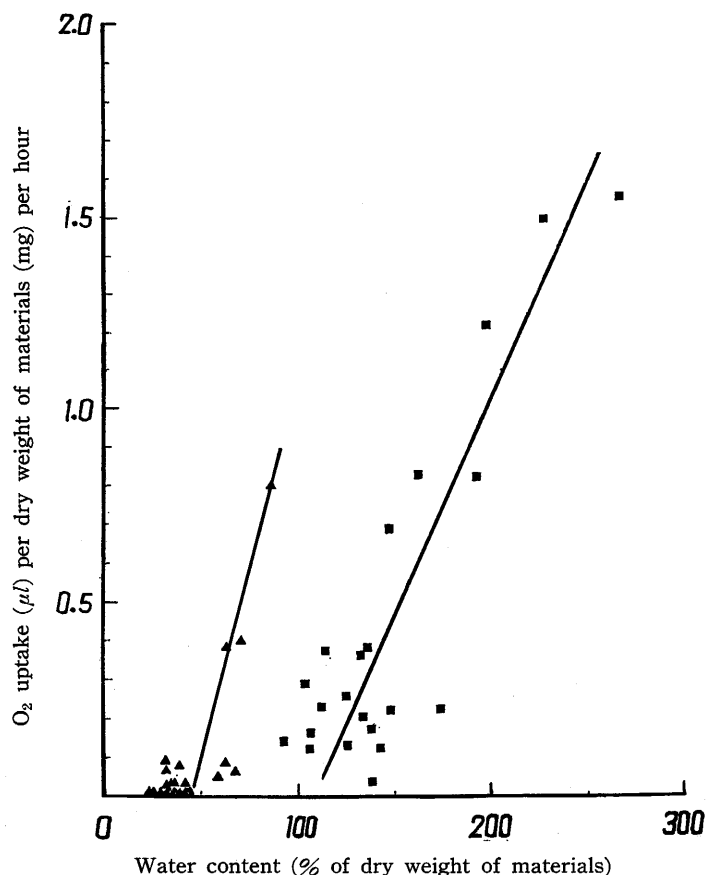


Fig. 6. Correlation between O_2 uptake and water content.

Filled squares: embryos; filled triangles: endosperms.

after dissection showed the appreciable increase at the first, then it fell down reversely. Fig. 4 indicates also a comparison of respiration on dry weight basis, and Fig. 5 and Table 3 do that of water content.

From these results, it is apparent that the embryo and endosperm of higher respiratory activity contain more water, as shown evidently in the decoated seed and that organs. Such a plausibility is shown in a correlation between O_2 uptake and water content in the isolated embryos and dissected endosperms respectively, calculated on the values in Figs. 4 and 5 (Fig. 6).

The effect of DNP on O_2 uptake of embryos is illustrated in Figs. 7 and 8. There was no significant difference of its inhibiting or promoting effect between the embryos isolated from stratified and unstratified seeds. But, it is likely that the addition of DNP promoted slightly the production of CO_2 in the embryo from unstratified seed (Table 4).

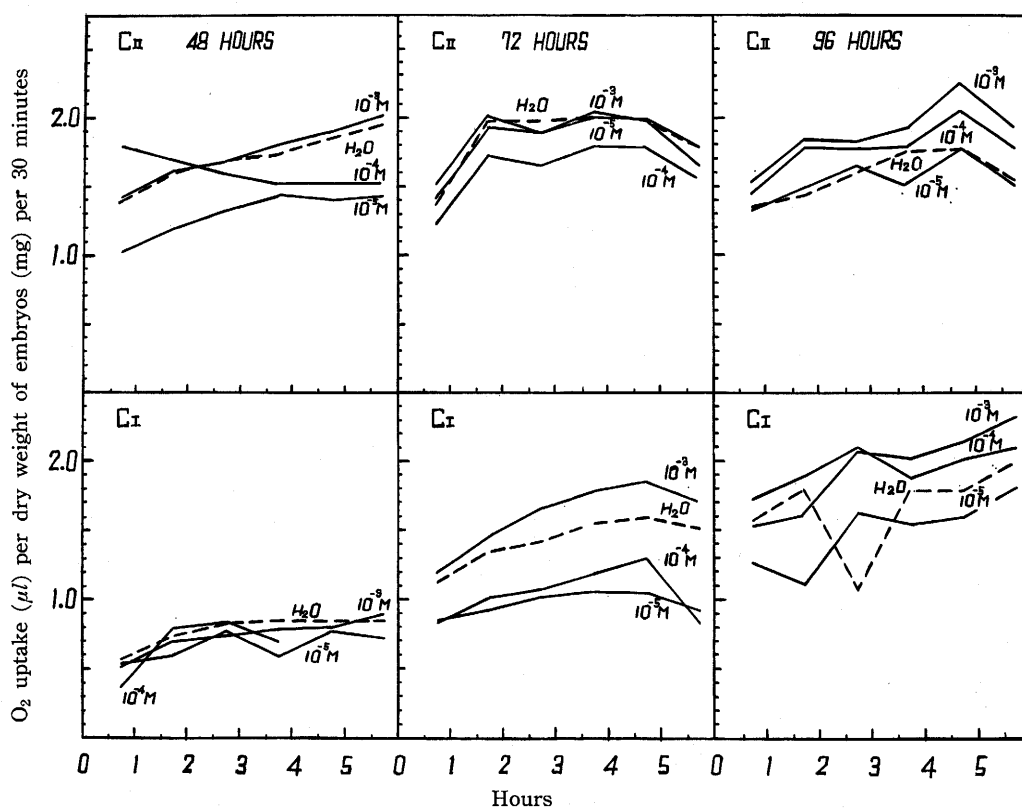


Fig. 7. Effect of DNP on O_2 uptake in the embryos isolated from decoated seeds.

C_I : unstratified seeds; C_{II} : stratified seeds. Hours in the figure: same in Fig. 4. The measurement was begun between 0.5 and 1 hour after the operation to isolate, with the start by DNP solution pour from the side chamber. Abscissa: same in Fig. 3. Concentration in the figure shows that of DNP, and pH values of its solution were each 3.8 in $10^{-3}M$, 4.6 in $10^{-4}M$, and 5.2 in $10^{-5}M$.

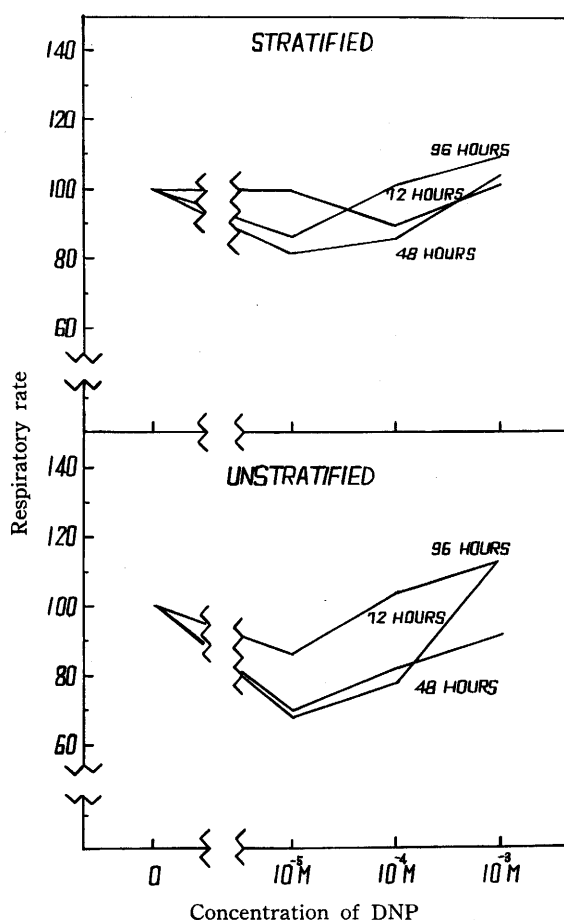


Fig. 8. Respiratory rate of the isolated embryos at various concentration of DNP. Respiratory rate indicates the rate of O_2 uptake (μl) per dry weight of embryos (mg) between 3.5 and 4.0 hours (30 minutes) as percentage of the control (with water). Hours in the figure: same in Fig. 3.

Table 4. Change of R. Q. values in the isolated embryos and dissected endosperms.

Incubation period of decoated seeds at 25°C (hours)	Unstratified						Stratified					
	embryo				endosperm		embryo				endosperm	
	with water		with DNP		with water		with water		with DNP		with water	
	A	B	A	B	A	B	A	B	A	B	A	B
0	1.03*	0.86	—	—	0.38*	0.63	1.01*	1.10	—	—	1.05	1.06
20	0.57	0.88	—	—	1.00	0.36	0.94	0.96	—	—	1.03	1.04
45~48	0.52	0.75	1.00	1.12	0.64	0.95	0.96	0.98	0.89	0.97	1.03	1.02
69~72	0.44	0.76	1.10	1.06	0.58	0.58	0.92	0.98	0.99	1.09	0.81	1.46
93~96	0.79	0.90	1.03	1.29	0.45	0.30	0.93	1.06	1.03	1.23	0.62	3.77

The mark (with DNP) shows the imbibition with 10^{-3} M solution of DNP.

A: values between 1 and 2 hours after the operation; B: between 5 and 6 hours.

The values with asterisks: between 3 and 4 hours.

Discussion

The results of these experiments show not only general feature in seed physiology, but also some characteristics with the germination of *Pinus koraiensis* seed.

As the pine seed has a thick and compact seed coat, the puncturing treatment, which is often favored, is not effective to its germination. The ability to take up oxygen and to absorb water could not be enhanced in the punctured seed, but the ability was elevated conspicuously in the decoated, stratified seed. It might be possibly stated that the seed coat would inhibit the swelling and respiring of cells in the endosperm and embryo, necessary to energy requirement of the embryo for the progress into the phase of activation and mitosis (EVENARI, 1957), and that the puncturing was not enough for the germination of such a seed, but the treatment resulted in inducing the attack of micro-organism during incubation of long period.

STANLEY (1957, 1958) has made a similar observation with stratification on the stimulated respiration of *Pinus lambertiana* seed. And, the activity to oxidize organic acids in mitochondria of the endosperm became higher with the imbibition, while the activity in the stratified embryo was rather reduced than in the unstratified embryo. His findings may accord in an explanation for the enhanced respiration in endosperm of this case. But, the enhanced respiratory activity and stimulated ability of water uptake in embryo should be explained by other factors.

The first consideration is directed to the change of physical status in cytoplasm, surrounding mitochondria. Stratification might affect the colloidal status through intracellular metabolism at low temperature, for example, the change of reserve substances from water-insoluble form into the soluble one, as suggested by the change of R.Q. values in Table 4. Thus the ability of oxygen- and water-uptake in embryo (and endosperm) might be enhanced by the physical change and some osmotic relations.

According to NYMAN (l.c.), the light-sensitive pine seed (*Pinus silvestris*) was germinated in darkness with stratification. Decoating and puncturing had a similar promotive effect on the germination. Respiration was also stimulated by these treatments.

The stimulation of respiration with DNP would be interested in relation to energy requiring process (Beevers, 1953; Millerd *et al.* 1953). POLLOCK and OLNEY (l.c.) had observed a high correlation between the stimulation of respiration with DNP and the accumulation of high energy phosphate in cherry seed organs, stratified at 5°C. Although from the present data, no significant difference with the effect of DNP on oxygen uptake in stratified and unstratified embryo was observed, more elevated R. Q. values in the unstratified embryos suggest the facility of turnover from aerobic respiratory mechanism into anaerobic mechanism with the uncoupler of oxidative phosphorylation in the embryo, and the rather slight change of the stratified embryo than in the unstratified embryo may show a reluctant turnover because of some relations with phosphate metabolism.

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Summary

Effects of stratification (at 0-5°C for 30 days) on the germination, respiration and water uptake were studied with *Pinus koraiensis* seed.

1) The prechilling treatment accelerated the germination of decoated and intact seeds, but it was not effective to that of punctured seed. Regardless of stratification or non-stratification, decoating promoted the germination of seed.

2) The seeds of rapid germination speed and their seed organs (isolated embryo and dissected endosperm), especially the decoated, stratified seed, showed higher ability to take up oxygen and to absorb water at the initial stage of germination.

3) Higher values of R. Q., indicated in the stratified seed organs, suggest the metabolic change of reserve substances during pretreatment, and by the addition of 2, 4-dinitrophenol the reluctant turnover of aerobic to anaerobic metabolism in the stratified embryo was alluded from the difference of R.Q. values in the stratified and unstratified embryos, that is, CO₂ production was elevated in the unstratified embryo.

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チヨウセンマツ種子の発芽, 呼吸ならびに 水分吸収におよぼす湿層処理の影響

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和 文 摘 要

チヨウセンマツ種子の発芽, 呼吸, 水分吸収におよぼす湿層処理 (0~5°C, 30 日) の影響を調べ, 次のような結果を得た。

1. 当処理の発芽促進効果は皮むきおよび無処理種子に認められ, ‘皮の傷つけ’ 種子には効果がない。湿層処理の如何にかかわらず, 皮むきは種子発芽に有効である。
2. 発芽速度の早くなった種子およびそれからの摘出胚, 切半した胚乳では発芽初期の酸素および水分吸収能力が高まる。
3. 湿層処理した胚, 胚乳の呼吸商 (R. Q.) の高まりは処理中の貯蔵物質の代謝を示唆し, また処理区および無処理区胚 両者間における 2,4-デニトロフェノール添加による呼吸商の差 (無処理区での炭酸ガス放出の高いこと) から, 処理区では, 有気呼吸より無気呼吸への転化が起こりにくくなっていることが暗示された。