Stimulating Effect of Mercuric Chloride and Silver Nitrate on the Dark Germination of Pine Seeds (II)

Ken-ichi HATANO*

CONTENTS

1. Introduction

It seems that there is little obstacle with the germination of *Pinus densiflora* seed for sylviculture practise in the forest nursery and the stand, because sun-light and daily alternating temperature possibly act on the germination favourably. However, when the seeds are sown and incubated at constant temperature in darkness, the germination percentage is generally low.

For the promotion of such germination, the pretreatment of mercuric chloride and silver nitrate has a specific stimulating effect, as described in the previous paper (HATANO, 1967).

Some assumptions are accounted for the mechanism of this stimulation, that is, the change of a sort of photomorphogenic receptor in darkness, the inactivation of growth inhibitors, the activation of some enzymes, or the physical denaturation of seed coats.

In this study, an approach to the mechanism is dealt with, in relation to inhibitors in the seed coats.

2. Materials and Methods

Pinus densiflora seed, used in this study, was generously supplied from the Forest Experiment Station of Ibaragi Prefecture. Storage of the seed in our laboratory, germination bed, dark condition, germination criterion, and germination percentage calculation were same as described previously (HATANO, 1967). The value of germination was also indicated by number of germinated seeds per 50. In all cases, the germination values were obtained as the average of two plots.

Safelight of green fluorescent lamp (10 watt) was used through green vinyl resin filter (Hishi Plate) with two layers of green celophan. Intensity of the illumination was around 10 lux at the level of seed handling. Other light sources were as follows:

white—white fluorescent lamps, 1,500 lux,

red—pink fluorescent lamps of Toshiba Co. through one layer of red vinyl resin filter, 300–400 lux, ca. 3,000 erg/cm^2. sec., maximum transmittance of the filter

* Address of the author: Tokyo University Forest Experiment Station, Tanashi, Tokyo.

was observed about 650 mμ.,

far-red—far-red lamps for medical use (Biolight, Toshiba Co.) through 10–14 cm water layer with each one layer of red, and blue vinyl resin filter, 2–3 lux, ca. 3,000 erg/cm². sec., maximum transmittance of the filter was obtained about 800 mμ.

Germination temperature was 23–26℃ in darkness, 26–28℃ in white light, and 25–27℃ in red and far-red.

The pretreatment of seed by heavy metal reagents, always followed with water cleansing for ten minutes, was carried out under the safelight or in darkness. In the case of addition of the chemicals to the seeds, they were applied to de-fatted cotton pieces, laid in PETRI dishes, so that the seeds were moistened appropriately.

The operation, to remove the seed coats (testa and papery membrane) and to puncture the coats by rubbing both tips of the seed on sand paper, was made before sowing the seeds (DECOATED and RUBBED, in figures).

For the measurement of oxygen uptake in seeds, the conventional WARBURG manometer was used under ordinary room light. Each fifty seeds, which were incubated previously at about 25℃ in darkness, were transferred from the germination bed on moistened filter paper in the main chamber of the vessel. Out put carbon dioxide was absorbed with 10% KOH in the center well. The measurement was started four hours after the equipment of the seeds. O_2 uptake was measured at 25±0.1℃ for three hours with 30 minute interval. Oscillation was 100 per minute. Duplicate values of O_2 uptake were averaged.

All these experiments were performed within one year and a half from receipt of the seed.

3. Results

The well-known stimulation of red illumination and inhibition of far-red were recognized in the germination of the pine seed (Table 1). And, the inhibition of far-red was released by the treatment of mercuric chloride or silver nitrate. The operation of seed coats stimulated also the dark germination (Fig. 1). Although the decoating opera-

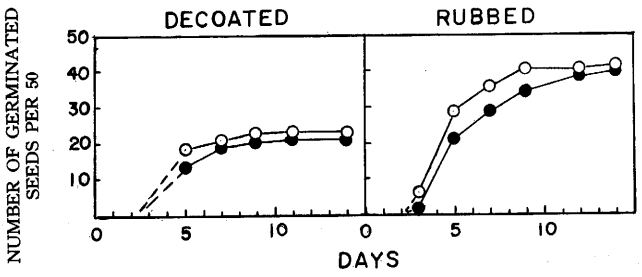

Fig. 1. Germination of decoated and rubbed seeds.
Empty circles: 24 hrs., illuminated by white fluorescent lamps (1,500 lux) at the initial stage, and then kept in darkness.
Filled circles: not treated.

Table 1. Effect of illumination and treatment of mercuric chloride and silver nitrate on the seed germination.

A

Germination % (20 days)			
Illuminated by			Dark
W	R	F R	control
55.3	54.7	2.0	24.2

B

Germination % (20 days)				
Treated with				Untreated
Ante-FR illumination		Post-FR illumination		
0.1% $HgCl_2$ 20 min.	0.1% $AgNO_3$ 5 min.	0.1% $HgCl_2$ 30 min.	0.1% $AgNO_3$ 10 min.	FR illumination
33.5	56.7	26.0	37.0	2.0

W: white, R: red, FR: far-red.
Illumination period was 24 hours at the initial stage of seed imbibition, and then kept in darkness.

tion had been very effective for the germination according to Goo (1965), the values in this study seemed to be modified by micro-organism's attack. The treatment of heavy metal solutions was not effective for the decoated seed, but the treatment of very short time periods was efficient for the rubbed seed (Fig. 2).

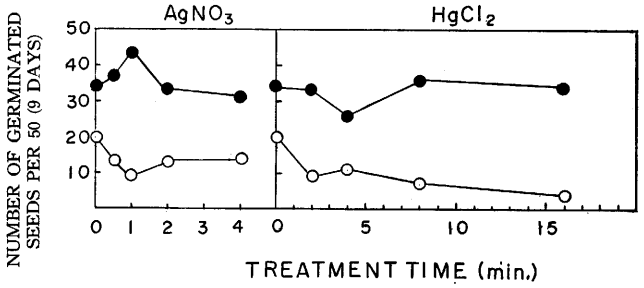

Fig. 2. Dark germination of seeds, treated with $AgNO_3$ and $HgCl_2$.
Concentration of $AgNO_3$ and $HgCl_2$ solutions was 0.1%.
Empty circles: decoated seed, filled circles: rubbed seed.

Table 2 shows the comparison of efficiency of the treatment with other favourable treatments to the dark germination.

As it was estimated that the seed coats contain coumarin and o-coumaric acid as phenolic compounds (HATANO, 1967), the effects and the release of silver nitrate toxicity by the phenolics were assayed (Table 3). In the chemicals as used in this experiment, coumarin (higher than 5×10^{-4} M) inhibited the germination of seed.* Coumarin or o-

* The radicle and hypocotyl of germinated pine seed grew abnormally dick and juicy in the coumarin solution.

Table 2. Comparison of mercuric chloride and silver nitrate treatment with other treatments.

A

Germination % (20 days)				
Pretreated with				Not treated
0.1% $HgCl_2$ 20 min.	0.1% $AgNO_3$ 5 min.	1% H_2O_2 24 hrs.	Washed with* water 24 hrs.	
58.6	59.8	47.7	46.2	24.2

B

Germination % (19 days)			
Prechilled at 2-5℃ for			Not treated
14 days	30 days	60 days	
77.5	94.5	94.0	15.5

* Treated in a well-cleansed small tank for photography development with deionized water in darkness. Germination bed was kept in darkness.

Table 3. Effect of silver nitrate and co-applied organic substances on the seed germination.

Germinated seeds per 50 (12 days)						
	Concentration (M) of applied $AgNO_3$					
	0	10^{-6}	10^{-5}	10^{-4}	5×10^{-4}	10^{-3}
Intact	9.0	12.5	20.0	24.5	20.0	6.0
Rubbed	33.0	28.0	31.0	33.5	4.0	10.0

Germinated seeds per 50 (12 days)				
Applied chemicals	Concentration (M)			
$AgNO_3$+Coumarin	0+0	$0+5\times10^{-4}$	$5\times10^{-5}+5\times10^{-4}$	$5\times10^{-4}+5\times10^{-4}$
Intact	9.0	5.0	10.5	27.5
Rubbed	28.0	19.5	18.5	5.0
$AgNO_3$+o-Coumaric acid				
Intact	9.0	9.0	20.5	32.0
Rubbed	28.0	28.0	22.5	5.0
$AgNO_3$+Oxalic acid				
Intact	6.5	12.0	18.0	19.5
Rubbed	29.5	34.5	24.0	22.5
$AgNO_3$+Citric acid				
Intact	6.5	8.5	19.5	25.0
Rubbed	29.5	34.5	29.5	33.0

Intact & Rubbed: same as described in Fig. 1.

coumaric acid, applied with silver nitrate together, worked indifferent or favourably on the intact seed. Oxalic and citric acids, which were used for comparison with coumarin and o-coumaric acid, acted easily to silver nitrate, accordingly the toxicity of silver nitrate seemed to be rather abated in both intact and rubbed seeds.

An aspect of respiration, as shown in Fig. 3, indicates that O_2 uptake in the seed, treated with mercuric chloride or silver nitrate, was suppressed during the germination stages before the emergence of elongating root from the testa.

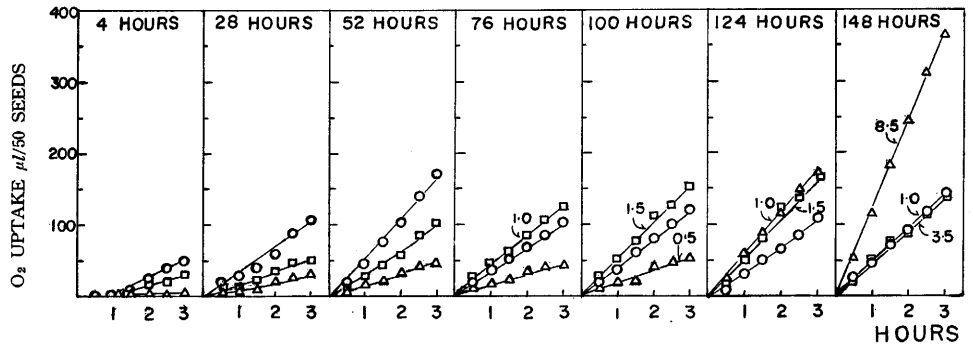

Fig. 3. Oxygen uptake of seeds, treated with $AgNO_3$ and $HgCl_2$.
Hours: time after the imbibition.
Circles: untreated, triangles: treated with $AgNO_3$ (0.1%) 5 min.,
squares: treated with $HgCl_2$ (0.1%) 20 min.
Numbers in diagram: germinated seeds per each 50.

4. Discussion

In the factors to regulate the dormancy of plant seeds, light seems to be often an important one to overcome other inhibiting factors. Red light breaks the dormancy and far-red blocks reversibly the progress of germination, as learned in the 'Phytochrome theory' (BORTHWICK and HENDRICKS, 1961; HENDRICKS, BUTLER and SIEGELMAN, 1962).

For the seeds of woody plants, however, light can not be the almighty promoting factor to break the dormancy. In some cases, the cause of such insufficiency should be attributed to the mechanical resistance of the seed coats to water uptake and the interior expansion (HATANO and ASAKAWA, 1964). In the case of *Pinus densiflora* seed (HASEGAWA and FURUKAWA, 1953) as like as *P. silvestris* (NYMAN, 1963), *P. virginiana* (TOOLE *et al.*, 1961), *P. taeda* and *P. strobus* (TOOLE *et al.*, 1962), it is not considered that the seed coats play a part of barrier with such function.

Red light stimulated the germination of *Pinus densiflora* seed, but it could not so elevate the germination over 90% level, as attained by pre-chilling or stratification. Far-red blocked the germination down to 2% level, and the treatment of mercuric chloride or silver nitrate released the far-red inhibition. Some system to control the dormancy other than the light-sensitive one of 'phytochrome' and/or high energy reaction (MOHR and APPUHN, 1963) should be supposed from the unsatisfactory elevation of the germination by red illumination and the efficiency of the heavy metal treatment

on the far-red inhibited seed.

From the results with the seed coat operation and the stimulation of silver nitrate in both decoated and rubbed seeds, the system might be related to the presence of inhibitors in the seed coats. Another support of the system would be found in the stimulation of germination with seed cleansing and hydrogen peroxide treatment, as shown in Table 2 (CHING, 1959).

It was possibly estimated that the inhibitors are phenolics as coumarin and o-coumaric acid. Only coumarin (5×10^{-4}M) inhibited the seed germination. Although it was obscure in this research whether the inhibition of coumarin was cleared by silver nitrate or not, there appeared a possibility that the phenolics change into the other substances with silver nitrate in the seed.

The suppression of oxygen uptake in Fig. 3 might be also concerned with the oxidative decomposition of phenolics. As silver nitrate or mercuric chloride, which remained in the seed coats, can be an oxidant, the uptake of oxygen may be diminished in the seed. The suppression would be of course explained by other possibility. The one is that the treatment of heavy metals suppresses the respiration of micro-organism in the superficial parts of the seed. Another one is that the heavy metals may play a part of controller for inhibitors or decompose the seed coat tissues gradually, but that the metal compounds remained in the tissues or the decomposing tissues may hinder gas exchange by blocking the perforation at the initial phase.

Recent studies have pointed out that light for seed germination is often substituted by gibberellin, kinetin, and other chemicals (MAYER and POLJAKOFF-MAYBER, 1963; TAKAHASHI, MOROO, HASHIMOTO and YAMAKI, 1962). There may be present the other function of the heavy metal compounds on the metabolic activity through the action for enzymes or growth substances.

Stimulation of silver nitrate was reported in the rooting of cutting in *Myrica rubra* and *Castanea crenata* (OOYAMA, 1962). There might be a relevant system of silver nitrate stimulation between the seed germination and the rooting of cutting.

5. Acknowledgements

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6. Summary

The dark germination of *Pinus densiflora* seed is stimulated by the treatment of 0.1% mercuric chloride or silver nitrate for short periods of time ante-sowing.

The treated seeds overcame the inhibition of far-red illumination to some degrees. This treatment was not effective for the decoated seed, but the efficiency was found in the rubbed seed, both tips of which was punctured by rubbing on sand paper.

The toxicity, that silver nitrate of higher concentration showed, was released by coumarin or o-coumaric acid rather in the intact seed. In both intact and rubbed seeds, oxalic or citric acid, which was used for comparison with the phenolics, abated the toxicity.

Oxygen uptake in the seeds, treated with mercuric chloride or silver nitrate, was suppressed before the germination.

From these results, the mechanism of the heavy metal stimulation was discussed in relation to the removal of inhibitors in the seed coats.

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昇汞および硝酸銀によるマツ種子の暗発芽促進 II (摘要)

文部教官　畑　野　健　一

アカマツ種子の暗発芽は0.1%昇汞水または硝酸銀溶液の短時間前処理によって促進される。処理された種子は赤外線照射抑制にもある程度打ちかった。この処理法は剝皮種子には有効でないが，種子の両端を紙ヤスリですって穴をあけた種子に有効であった。濃度の高い硝酸銀が示す毒性は，もとのままの種子に，クマリンやo-クマール酸添加によってゆるめられ，シュウ酸やクエン酸はすった種子およびもとのままの種子両者にてその毒性を減少させた。昇汞や硝酸銀で処理した種子の酸素吸収は発芽前おさえられる。

これらの結果をもとに，抑制物質の除去と関係して，これら重金属による発芽促進のメカニズムを論じた。