

The effects of ethyleneimine and ethyl methanesulphonate on the germination of *Pinus rigida* seed

by K. B. Yim*

The mutagenic action of ethyleneimine ($\text{CH}_2\text{CH}_2\text{NH}$) in barley has been reported by Ehrenberg, et al., (1958). To permit comparison with radiation effects, the concentration (dose) giving 50 % lethality was set equal to unity on a linear scale. According to their results, when the mutation rate was estimated from the frequency of spikes segregation for chlorophyll deficient seedlings the ethyleneimine was 3-5 times more efficient a mutagenic agent than X-rays. Some other chemicals have also been investigated, with a view to the possibilities of influencing the mutation process (Ehrenberg, et al., 1956; Ehrenberg and Gustafsson, 1957).

Mutation rates, of a magnitude never reached with ionizing radiations, have been obtained in barley by ethylene compounds, i. e., ethylene oxide ($\text{CH}_2\text{CH}_2\text{O}$) and ethyleneimine (Ehrenberg, et al., 1959). Blixt, et al., (1960) found ethyleneimine of high mutagenic efficiency in the pea, variety *Weitor*. They found that the mutation rate was correlated with uptake of solution and the frequency of leaf spots on the first leaves. In addition, germination and growth rate of seedlings, survival and branching of the plants exhibited a similar correlation, while at low concentrations ethyleneimine stimulated germination and growth. A comprehensive literature review on chemical mutagenesis in higher plants has been made (Gustafsson, 1960).

In the present paper the effects of mutagenic agents, ethyleneimine and ethyl methanesulphonate ($\text{CH}_3\text{SO}_3\text{C}_2\text{H}_5$), on the germination of *Pinus rigida* (pitch pine) seeds are analysed. In addition, the equivalent radiation doses of neutrons, X-rays and gamma-rays which cause the same degree of germination depression are given for comparison.

Materials and Methods

Pitch pine seeds belonging to Seed-Size-Class 3

were used, to avoid experimental error due to variations in seed size. The present author has made the following standard Seed-Size-Class as applied to pine seeds. Sorting the pine seeds into each Seed-Size-Class can easily be made with a set of sieves.

Seed-Size-Class	1	2	3	4
The range of maximum width of seed (mm)	3.5-3.1	3.0-2.6	2.5-2.1	2.0-1.6

Two hundred seeds were soaked in 1.0 ml of ethyl methanesulphonate (M.W. = 124.16, Sp. Gr. = 1.3) and ethyleneimine (B.P. = 55-56°C., Sp. Gr. = 0.8) solution of appropriate concentration contained in glass tubes. All glass tubes were put on an electric shaker during treatment and shaken for 3 hours in ethyleneimine solution when not neutralized and for 6 hours if neutralized, and also when in ethyl methanesulphonate solution. The experimental design adopted was a randomized complete block arrangement with four replications, 50 seeds in each treatment unit. After completion of treatment, the seeds were carefully washed with tap water and sown in petri-dishes, in the bottom of which were put two sheets of water retaining filter paper. This experiment was carried out in 1961 at the Institute of Forest Genetics, Stockholm 51.

Results and Discussion

(1) The effect of ethyleneimine solution treatment of pitch pine seeds on subsequent germination.

(a) When the solution was not neutralized with HCL.

The seeds were sown on May 9, 1961, and the germination rate was counted at 10 am. every day. The observed germination sequences are presented in Fig. 1.

As seen from Fig. 1, the control seeds showed normal germination, but the germination of seeds

*Associate Professor, College of Agriculture, Seoul National University, Suwon, Korea

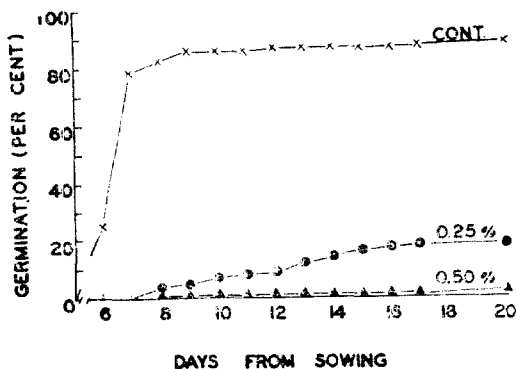


Fig. 1. Correlation between germination percentage on successive days from sowing of pitch pine seeds presoaked in ethyleneimine solutions. The solution was not neutralized.

treated in 0.25 per cent solution was, however, severely depressed. When the concentration exceeded 0.5 per cent, almost complete death of seeds resulted. This possibly is due to the high pH value of the solution, as the germination and growth of conifer seeds is not favoured by an alkaline growth medium. This was later demonstrated by neutralizing the solution with 1 N HCL. In control seeds, maximum germination was reached 7 days after sowing, but in treated seeds, this was uncertain within the period of the investigation.

(b) When the solution was neutralized with HCL.

The results presented in Fig. 2 indicate that even

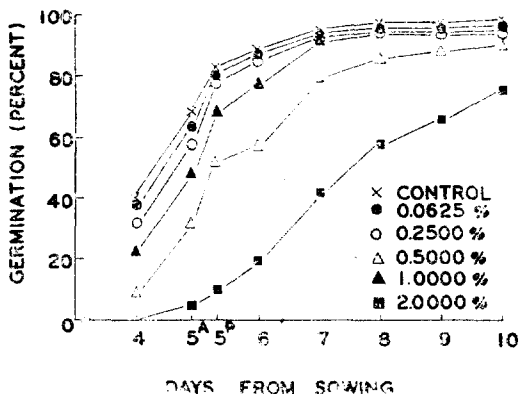


Fig. 2. Correlation between germination percentage on successive days from sowing of pitch pine seeds presoaked in ethyleneimine solution. The pH of the solution was neutralized with 1N HCL. 5a: at 10 am and 5p: at 8 pm on the 5th day from sowing.

seeds treated with 1.0 per cent solution showed about 90 per cent germination 10 days from sowing, contrary to the results of the previous test. At lower concentrations, maximum germination was reached 7 days from sowing, as with the control seeds. In the case of seeds treated with higher concentration, i. e., 2.0 per cent, the number of seeds germinating gradually increased until 10 days from sowing.

(2) The effect of ethyl methanesulphonate solution treatment of pitch pine seeds on subsequent germination.

The seeds were sown on June 11, 1961. The germination results are presented in Fig. 3. It is apparent that there was a delay in the germination of treated seed, but afterwards with the 0.25 and 0.50 per cent treatments, germination rapidly increased. This tendency is contrary to that of ethyleneimine.

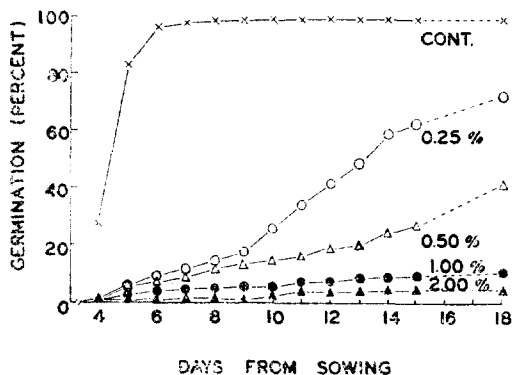


Fig. 3. Correlation between germination percentage on successive days from sowing of pitch pine seeds presoaked in ethyl methanesulphonate solution of various concentrations.

The germination toxicity of ethyl methanesulphonate is weaker than that of ethyleneimine which is not neutralized, but it is however stronger than that of neutralized ethyleneimine. In dry barley seeds, the concentration of ethyleneimine solution causing 50 % death was 0.060 per cent (Ehrenberg, et al., 1958). This value is lower than that for dry pitch pine seeds, i. e., 1.89 per cent. The planting method, the place where the seeds were sown and the length of periods between sowing seeds and calculating the results might account for this.

In order to illustrate the decreasing germination capacity with increase in concentration of the solution,

Figures 4, 5 and 6 present the data as percentage of the control.

For parallel test, neutron irradiation was conducted at the atomic reactor, RI, in Stockholm. Here, the densely ionizing radiations are produced by fast neutrons plus epithermal neutrons. The total dose rate of densely ionizing radiations in the vertical

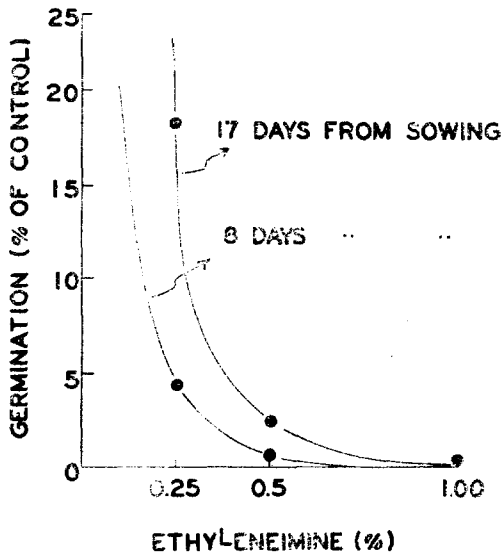


Fig. 4. The germination percentage of pitch pine seeds pretreated with ethyleneimine solution without neutralization (as per cent of control).

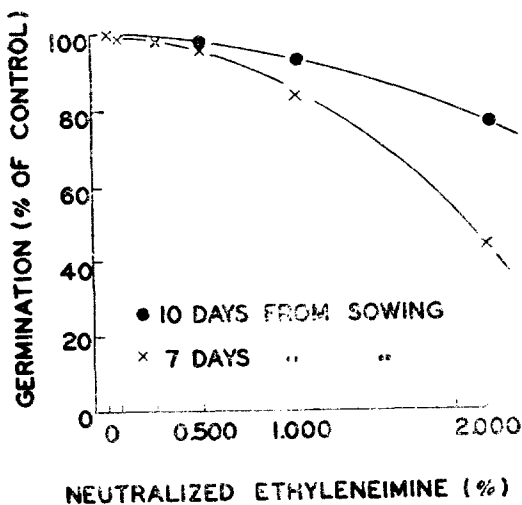


Fig. 5. The germination percentage of pitch pine seeds pretreated with neutralized ethyleneimine solution (as per cent of control).

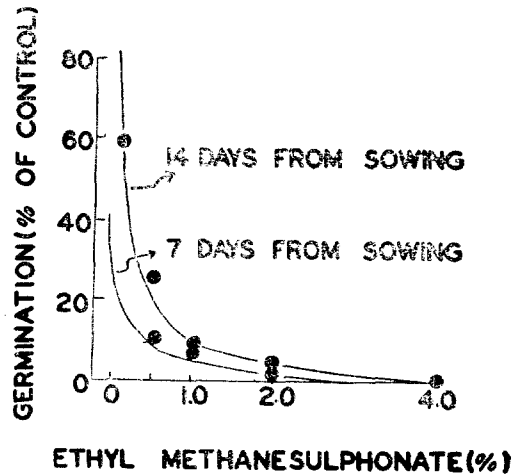


Fig. 6. The germination percentage of pitch pine seeds pretreated with ethyl methanesulphonate (as per cent of control).

channel leading through the center of the reactor was about 0.7 rad/Sec. Gamma-ray irradiation was made at the Institute of Organic Chemistry and Biochemistry, Univ. of Stockholm. The dose rate produced from the Co-60 source was 8100 r/min. X-ray irradiation was made at the Institute of Forest Genetics, Stockholm 51., with focus 30 cm., filter 0.5 mm/cu+1 mm/al., 200 Kv. and 15 mA.

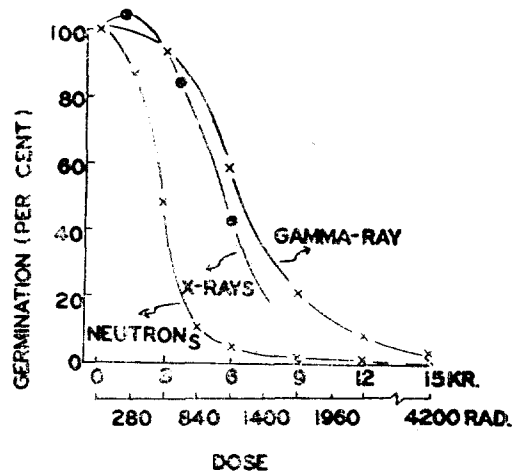


Fig. 7. Correlation between germination percentage of pitch pine seeds and radiation dose at 7 days from sowing when the maximum germination energy of the control seeds was reached.

Table 1 was obtained from Figs. 4-7 and presents data on the equivalent irradiation doses causing the

same level of germination suppression as the chemical mutagens. The data are based on the time when the maximum germinative energy of control seeds was reached. The LD-50 is given for neutralized

ethyleneimine solution and LD-80 in other cases.

Table 1. Comparative lethal concentration (doses) of mutagenic agents in terms of suppression of pitch pine seed germination.

level of LD(%)	equivalent concentration (dose) of					
	Ethyleneimine	Neutralized ethyleneimine	Ethyl methane sulphonate	Neutron (rad)	X-rays (Kr.)	γ -rays (Kr.)
LD-80 1/	0.10%	—%	0.12%	700	7.5	9.2
LD-50 1/	—	1.89	—	550	5.7	6.5
LD-80 2/	0.25	—	0.50	—	—	—
LD-20 2/	—	1.15	—	—	—	—

1/ When maximum germinative energy of control seed was reached.

2/ When maximum germination expressed as percent of control seed was reached.

Summary

1. Almost complete death of pitch pine seeds resulted when presoaked in 0.5 per cent ethyleneimine solution without neutralization. When neutralized with 1 N HCL, quite high germination percentages were obtained even with 2 per cent solution.
2. When presoaked in 0.25 or 0.50 per cent ethyl methanesulphonate, there was a delay in germination, but the germination per cent rapidly increased from about the 10 th day from sowing.
3. Basing upon the time when the germinative energy of control seeds was reached, the comparative lethal doses (concentrations) among mutagenic chemicals and radiations were investigated (Table 1).

1.89 per cent ethyleneimine solution with neutralization, 550 rads of neutron, 5.7 Kr. of X-rays and 6.5 Kr. of gamma-rays gave the same effect on killing 50 per cent of seeds.

Literature cited

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Ethyleneimine 과 Ethyl methanesulphonate 가 리기다소나무 種子의

發芽에 주는 影響, 特히 放射線照射量과의 效果比較

서울大學校 農科大學 任 慶 彬

本研究의 結果를 다음과 같이 要約한다.

1. 中和시키지 않았는 0.5% Ethyleneimine 水溶液에 處理된 pinus rigida 의 종자는 거의 致死되나 1 N HCl 로 中和가 되면 相當한 發芽를 보이며, 농도가 2%에 達해도 아직 높은 發芽率이 얻어진다.
2. 0.25% 또는 0.50%의 ethyl methansulphonate 의 水溶液에 Pinus rigida 種子를 處理하였을 때에는 初期發芽에 遲滯가 招來되나 10日 以後에 가서는 發芽가 旺盛하게 되었다.
3. 對照區種子의 germination energy가 얻어졌을

때의 값을 기준으로 해서 分析하면 表 1에 보인 것과 같은 이곳 mutagenic chemicals 와 放射線의 比較 致死量이 얻어졌다. 즉 中和된 1.89%의 ethyleneimine 의 水溶液, 550 rads 의 中性子照射量, 5.7 kr 의 X線 그리고 6.5 kr 의 gamma線은 같은 致死效果 LD₅₀를 주었다.

4. 이 試驗은 이곳 化學藥劑가 特有한 性質을 갖은 突然變異體의 誘起劑이고 또 放射線이 그와 같은 目的으로 使用되므로 그 效果를 究明하고자 遂行된 것이다.