dieser Bäume enthielt für ein rezessives Gen homozygote chlorotische Sämlinge.

Man kann aus den Untersuchungen schließen, daß *Pinus resinosa*, sowohl Einzelbäume wie die ganze Art, für eine große Anzahl Allele homozygot ist, daß sie selbstfertil und selbst-compatibel ist und daß die Sämlinge aus Selbstungen nur geringe oder gar keine Inzuchtdepression zeigen.

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Effect of the Chemical Mutagen Ethyl Methanesulfonate on Western White Pine

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Introduction

Induced mutation as a method of increasing genetic variability in trees has so far been neither profitable nor practical. This is largely because of the length of time required to obtain segregating second and later generation (M_2, M_3, etc.) progenies and to screen for genetic mutants. Also, the availability of large, virtually untapped pools of variation obtainable from natural populations and hybridization has inhibited research in use of induced mutation.

Disease resistance is one characteristic that holds considerable promise for improvement by induced mutation (Bhatia *et al.*, 1961; Konzak, 1956; Sparrow and Konzak, 1958). In systems involving hosts and obligate parasites, such as the rust fungi, a delicate physiological relationship exists. Mutations causing a subtle alteration in the host's physiology could be potential sources of genetic resistance. In addition, rust resistance is often a relatively simply inherited character (Stevenson and Jones, 1953). Therefore, it may be amenable to improvement through selection of resistant mutants.

The U. S. Forsst Service has a long-range program aimed at genetic control of the blister rust fungus, *Cronartium ribicola* A. Fisch. infecting western white pine (*Pinus monticola* Dougl.). Progress has been made towards production of resistant varieties by family selection (Bingham *et al.*, 1953; Bingham *et al.*, 1960). The number of selected trees that have been progeny-tested and reselected on the basis of general combining ability for a fair level of resistance is now small. Researchers expect that about 25 reselected parents will be available in each of three seed orchards. In addition to this within-species breeding program, work has begun to transfer the inherent resistance of certain species to western white pine through interspecific hybridization. The present study is the first effort to use in-

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duced mutation to search for sources of blister rust resistance.

Certain chemical mutagens result in low rates of chromosome breakage and high rates of gene mutation in barley (Heiner *et al.*, 1960). Thus, chemical mutagens might be quite effective in bringing about a physiological change to produce rust resistance without producing undesirable gross chromosomal changes. EMS was selected as the mutagen for this study because of its demonstrated effectiveness in inducing mutations in barley (Ehrenberg *et al.*, 1961; Froese-Gertzen, 1962; Konzak *et al.*, 1961; Swaminathan *et al.*, 1962). Nothing was known about its effect on seed of any tree species.

This study had two objectives: (1) to determine the physiological effects of EMS, ethyl methanesulfonate ($CH_3CH_2OSO_2CH_3$), on seed and seedlings and (2) to induce genetic resistance against the blister rust fungus. The latter is a long-term objective, and progress toward this goal must await resistance tests on progenies from crosses between M_1 plants. Only effects on seed and seedling physiology can be reported at this time.

Methods and Materials

Sound seeds, representing eight progenies produced by controlled pollination, were selected to test for genetic variation in sensitivity to chemical treatments. All seeds were stratified in peat moss at 5^0 C. for at least 4 months before treatment. Three hundred and sixty seeds from each progeny were divided into 12 lots of 30 seeds. Each lot was placed in a 125 ml. Erlenmeyer flask. Immediately before treatment each lot was thoroughly washed with distilled water. Then it received one of four treatments:

1 — 0.05 molar EMS — 4 hours
2 — 0.10 molar EMS — 4 hours
3 — 0.10 molar EMS — 8 hours
4 — Control — water (no chemical) — 4 hours

Each treatment was replicated three times for a progeny. All flasks containing seed and solution were agitated throughout the treatment period. After the EMS solution was decanted the lot of seed was rinsed twice with water and soaked in water for 45 minutes.

After treatment, the seeds were immediately planted in metal flats (14 by 20 by 3-3/4 inches). The flats contained a homogeneous mixture of sandy loam forest soil, decomposed sawdust, and coarse sand. Each seed was hand-planted; a template was used to assure a uniform 3-inch spacing between rows and ½-inch spacing between seeds within rows. The 30 seeds of a lot were planted in each row. A template numbered 1—30 made it readily possible to identify and record all observations on each seedling during the test. The seeds were planted on top of the soil, then covered with a uniform layer of fine sand. During the entire period of observation, the flats were kept in a greenhouse under a controlled temperature of about 21^0 C. and under a 16-hour photoperiod. By restricting the soil moisture level to the optimum required for germination, loss of seedlings to damping-off fungi was almost eliminated.

Each flat was examined daily, and the date at which each seed emerged was recorded along with any abnormalities. Also, the date the cotyledons expanded was noted. Hypocotyl length, cotyledon length, and the number of cotyledons were recorded 50 days after sowing for each seedling. All seed that failed to germinate were exhumed and dissected to determine how many were obviously decayed. A mean value was calculated for each characteristic in each treatment (*table 1*). These values represent combinations of all crosses and replications. The data were subjected to analysis of variance to determine the effectiveness of the chemical mutagen in modifying the characters studied. Values for percentage of germination were transformed to angles (angle = arcsine $\sqrt{\text{percentage}}$).

Table 1. — Average effect of treatment and progenies on each characteristic.

	Characteristic					
	Germination	Germination time	Cotyledon opening time	Cotyledons	Cotyledon length	Hypocotyl length
	Percent	Days	Days	Number	Mm.	Mm.
Treatment						
Control	73.2	10.0	8.8	8.2	27.0	30.6
0.05 m/4 hrs.	74 2	9.9	9.2	8.2	27.2	30.0
0.10 m/4 hrs.	67.9	11.0	8.9	8.1	26.9	29.2
0.10 m/8 hrs.	54.0	11.9	9.4	8.2	26.9	29.7
Progenies						
Selfs	52.8	12.6	8.9	8.0	24.1	27.3
Outcrosses	61.1	10.1	9.2	8.2	27.9	30.8

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	F-values					
		Germination	Germination time	Cotyledons open	Cotyledons	Cotyledon length	Hypocotyl length
		Percent	Days	Days	Number	Mm.	Mm.
Among progenies	7	87.39***	14.45***	13.78***	1.76	10.58***	42.08***
Selfs vs. outcrosses 1		115.02***	32.81***	5.96*	2.44	183.03***	74.14***
Among treatments	3	282.68***	5.90**	8.63***	0.40	0.26	2.72
Treated vs. controls 1		15.19***	4.63*	13.48***	0.20	0.00	5.45*
Crosses × treatments	21	0.63	1.27	1.37	0.10	0.92	0.61
Error	64						
Total	95						

* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.
*** Significant at the 0.001 level of probability.

Results and Discussion

Four characters were significantly affected by treatment: percent germination, germination time, hypocotyl length, and time of cotyledon opening *(table 1)*. Germination percent decreased as concentration and time of chemical treatment increased. Time required for seeds to germinate increased as treatment increased. Cotyledon opening time was delayed as treatment increased. Length of hypocotyl was less as treatment increased. All characteristics show a pronounced negative effect of the concentration and time of treatment on these growth phenomena. Cotyledon number undoubtedly was not affected because this character is determined long before seed ripening.

Progenies varied significantly in all characteristics except number of cotyledons *(table 1)*. Selfed progenies differed from outcross progenies in that they had a lower average percent germination, longer germination time, shorter cotyledon opening time, shorter hypocotyls, and shorter cotyledons. Thus the average effect of selfing on these characteristics was negative and similar, except for cotyledon opening time, to the effect of increasing chemical treatment.

The significant variance of progenies with respect to five of six attributes indicates either seed weight or inherent influences on these characteristics. To eliminate these two factors as sources of variation obscuring effects of treatment, separate analyses of variance were made within each cross. Treatment did result in growth depression, but the effect varied between crosses *(table 2)*. The *treatment × cross* interaction was not significant. Apparently selfed progenies are less subject to physiological damage by the chemical mutagen than are outcrossed progenies.

Number of cotyledons and cotyledon length might be expected to be least affected by a relatively mild chemical treatment. Neither trait varied significantly within any of the progenies *(table 2)*. Some progenies showed a significant variation in cotyledon length not associated with treatment *(table 2)*. A comparison of seed weight and cotyledon length showed these two traits to be positively correlated, at least for the few progenies in this study.

Neither seed decay nor seedling survival, approximating 100 percent, were significantly related to treatment. Upon completion of the 50-day greenhouse test, all surviving seedlings and soil were removed as a unit from each flat and planted in a nursery plot at Moscow, Idaho. One plant examined during the second year of growth exhibited somatic mutations. This plant came from a seed that had received the highest level of EMS treatment. The mutant had five fascicle bundles that contained needles which varied in color and size. One bundle had five yellow, short needles; two bundles had five yellow, normal-sized needles; one bundle had four normal-sized and two short needles, all normal green; and one bundle had two green, one yellow, two half-yellow, half-green (longitudinally) normal-sized needles, and two short needles, one yellow and one green.

If a beneficial genetic mutation in cells of white pine seeds were to result from the mutagenic action of ethyl methanesulfonate, it would be desirable that no depression of height growth be associated with the mutation. The results presented here indicate that EMS does penetrate to the embryonic tissue of pine seeds where it could function as a mutagen. Its depressing effect on many of the physiological responses described here combined with only slight overall early growth depression is encouraging.

Abnormal seed germination, usually associated with one of the EMS treatments, was common during this experiment. This included complete failure of cotyledon development, double seedlings, and reversed embryos. Such aberrancies have been observed previously and were not given further attention here (Schubert, 1955; Black, 1960).

All of the surviving seedlings in this study will be kept for observation and future production and testing of M_2 generations for mutations some 6 to 10 years hence. Meanwhile, the possibility of treating pollen (Neuffer and Ficsor, 1963) with this and other mutagenic agents to eliminate a wait of one generation should be explored.

Summary

The chemical mutagen ethyl methanesulfonate (EMS) was applied in three different concentration-time combinations to control-pollinated western white pine seed after stratification. Eight progenies, including two from self-pollination, were compared for response to the treatments. EMS significantly affected percentage of germination, time of germination, time of cotyledon opening, and hypocotyl length. Number of cotyledons and cotyledon length were not affected. Variations in percentage of germination, germination time, and time of cotyledon opening indicated that individual progenies responded differentially to treatment. Self-pollinated progenies appeared less prone than outcrossed progenies to physiological damage by the mutagen. Examination of the second year's growth on all seedlings revealed one plant with somatic mutations. These included yellow and green striped needles, yellow needles,

Table 2. — Significance of variance in characteristics due to chemical treatment within progenies.

Progenies 1)	F-values					
	Germination	Germination time	Cotyledons open	Cotyledons	Cotyledon length	Hypocotyl length
	Percent	Days	Days	Number	Mm.	Mm.
158 × 17	2.24	5.65*	0.22	0.45	0.79	3.06
166 × 58	7.19*	4.65*	4.73*	0.13	2.49	0.65
169 × 22	4.17*	5.76*	3.62	0.66	0.24	0.63
58 × 158	4.24*	2.47	3.50	0.00	1.29	0.16
68 × 17	5.25*	1.47	8.66**	1.32	1.42	0.55
94 × 17	7.63**	1.59	0.00	1.32	0.22	0.27
158 × 158	2.79	1.35	1.38	2.70	0.75	0.38
68 × 68	1.60	0.22	4.96*	1.33	0.39	1.71

* Significant at the 0.05 level of probability.

** Significant at the 0.01 level of probability.

1) Numbers refer to female and male parents, respectively, for each cross.

dwarfed needles, and variation in number of needles per fascicle sheath.

Zusammenfassung

Titel der Arbeit: *Über die Wirkung des chemischen Mutagens Äthylmethansulfonat (EMS) bei Pinus monticola.*

Mit dem chemischen Mutagen EMS wurde in 3 verschiedenen Konzentration-Zeit-Kombinationen stratifiziertes Saatgut von kontrolliert bestäubten *Pinus monticola*-Kiefern behandelt. Es wurden dabei 8 Nachkommenschaften, einschließlich 2 Selbstungen, hinsichtlich Behandlungseffekten verglichen: EMS wirkte signifikant auf das Keimprozent, die Keimdauer, die Dauer bis zum Spreizen der Kotyledonen und auf die Hypokotyllänge. Die Anzahl und die Länge der Kotyledonen wurden nicht beeinflußt. Die Variation im Keimprozent, der Keimdauer und der Zeit bis zum Spreizen der Kotyledonen zeigte, daß die einzelnen Nachkommenschaften verschieden auf die Behandlung reagierten. Selbstungsnachkommenschaften schienen hinsichtlich der durch das Mutagen ausgelösten Schäden weniger beeinflußt als Kreuzungsnachkommenschaften. Die Untersuchung im 2. Wachstumsjahr der Sämlinge ergab eine Pflanze mit somatischen Mutationen. Sie besaß gelb-grüngestreifte Nadeln, gelbe Nadeln, extrem kleine Nadeln und veränderte Nadelzahlen je Nadelbündel (Faszikel).

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Newsletter

Philip C. Wakeley, widely recognized authority on the regeneration and genetics of southern pines, will retire in October from the Forest Service, U. S. Department of Agriculture. He will have completed 40 years of Federal service, almost all of it at the Southern Forest Experiment Station in New Orleans.

In announcing Mr. Wakeley's plans for retirement, W. M. Zillgitt, Director of the Southern Station, said, "The work of this dedicated scientist is a source of pride for the Station, the Forest Service, and the Department of Agriculture. His contributions to forestry will be of lasting benefit to the timber economy of the entire South. His work during his distinguished career has affected — directly or indirectly — almost every one of the 11 million southern acres planted to pine since 1935."

Mr. Wakeley anticipated by more than 35 years the current widespread interest in forest genetics. In the fall of 1925 he obtained seed for study of geographic races of loblolly pine, the results of which proved that using seed from the wrong source could reduce returns from loblolly plantations as much as sixty percent. In the spring of 1929 he artificially hybridized longleaf and slash pines; this was the first recorded controlled cross of southern pines, and is believed to be the third authentic controlled cross ever made with any pines. Since 1951 he has devoted much of his time to coordinating a cooperative study, by about sixty agencies and individuals, of geographic variation of four species of pines; the plantations in this study include 136,000 trees at various points in 16 southern States.

Mr. Wakeley has written more than 80 publications that have furnished basic information for southern reforestation and tree improvement projects. His 1935 publication, "Artificial Reforestation in the Southern Pine Region" (USDA Technical Bulletin 492), guided the expanding State and Federal tree planting under CCC programs. In 1954, his USDA Agriculture Monograph 18, "Planting the Southern Pines", was issued; it quickly became the authoritative reference for southern reforestation.

In 1955, Mr. Wakeley received the U. S. Department of Agriculture's Superior Service Award. In 1956, the Society of American Foresters presented to him its Award for Achievement in Research.

Mr. Wakeley studied forestry at Cornell University, receiving the Bachelor of Science degree in 1923 and the Master's degree in 1925. He is a Fellow of the Society of American Foresters, a Fellow of the American Association for the Advancement of Science, and a member of the Ecological Society and the Biometrics Society. He is past chairman of the Society of American Foresters' Division of Silviculture, and former associate editor of the *Journal of Forestry.*

After October, Mr. and Mrs. Wakeley will make their home in Ithaca, New York.