A FIELD TEST OF DOUGLAS-FIR, PONDEROSA PINE, AND SUGAR PINE SEEDS TREATED WITH HYDROGEN PEROXIDE

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Hydrogen peroxide can speed germination of many conifer seeds. This capability has been used in the laboratory and might also promote fast germination in the field. The experimental sowing described provides clues about possible field use of peroxide-treated seed.

Background

Prior to the research reported here, hydrogen peroxide had been used on conifer seeds to achieve one or more of three objectives: (1) To rapidly test seed viability, (2) to surface sterilize seed used in cultures, and (3) to obtain seedlings quickly for experimental trials.

Ching and Parker (1958) first reported using weak hydrogen peroxide solutions to rapidly test the conifer seed viability of Douglas-fir, ponderosa pine, sugar pine, Jeffrey pine, lodgepole pine, noble fir, grand fir, and white fir. They opened the radicle end of each seed prior to immersion and germination in dilute hydrogen peroxide solution.

Others had reported earlier that water absorption and germination of Pinus densiflora and Cryptomeria japonica were affected when seeds were immersed in such solutions (Migita and Kawana 1953; Migita, Kawana, and Takahashi 1956a, 1956b).

Treatment with dilute solutions of hydrogen peroxide before or during germination on moist media has been tested on whole seeds of Douglas-fir, subalpine fir, and western larch (Ching 1959; Dhillon 1961; Dhillon and Johnson 1962; Schmidt 1962; Shearer and Tackle 1960), loblolly and slash pine (Carter and Jones 1962; Forrest 1964), and eastern white pine (Kozlowski 1962; Schmidt 1925). In most of these investigations, aimed primarily at finding simpler and faster tech niques for overcoming dormancy or determining seed viability, favorable seed response to hydrogen peroxide treatment was demonstrated.

Observations on the development of seedlings germinated from seed treated with hydrogen peroxide are found in three reports. Ching and Parker (1958) illustrated 3-weekold Douglas-fir seedlings developed from seed which had its radicle ends removed. Trappe (1961) reported that brief soaking of conifer seeds in 35 percent hydrogen peroxide caused no noticeable abnormality in later seedling development. Dhillon and Johnson (1962) found that hypocotyl growth from peroxide-treated seed was significantly less than that from stratified seed when larch seedlings were grown for 18 to 22 days after gemination in vermiculite-filled boxes. These three laboratory studies provided no evidence on the value of treating seed destined for field sowing with hydrogen peroxide.

Test Procedures [1]

The objective of the field trial reported here was to learn if treatment with hydrogen peroxide would speed germination of Douglasfir, ponderosa pine, and sugar pine. If brief soaking of dry seed in hydrogen peroxide solution speeded germination, this method might be substituted for the normal stratification or overwintering in the field usually required for good germination of these species.

Seeds of Douglas-fir, ponderosa pine, and sugar pine collected in the upper South Umpqua River drainage, Douglas County, Oreg., were used. Douglas-fir and ponderosa pine seeds had been in cold.storage near 0^{0} F. for about

1 The help of Frank A. TerBush, Jr., Umpqua National Forest, during initial phases of this study is gratefully acknowledged.

3 years; sugar pine seeds had been stored in a cool, unheated building through one winter only.

Seeds were soaked in water or in a 1-percent hydrogen peroxide solution at room temperature for 48 hours. The volume of liquid supplied was sufficient to provide 1, 1.5, or 2 milliliters per individual seed for Douglas-fir, ponderosa pine, and sugar pine, respectively. After 48 hours, seedcoats of Douglas-fir and ponderosa pine seeds soaked in hydrogen peroxide solution were bleached to a substantially lighter color; sugar pine seedcoats showed little bleaching.

Equal numbers of water- or peroxidesoaked seeds were sown in a screen-covered, 4- by 8-foot seedbed located on a water-laid pumice soil of loamy sand texture at Glide, Oreg. Mid-April, a relatively late sowing date, was chosen deliberately. By this time, germination of winter-sown seeds of the same species and origin was in progress in adjacent beds of another study. Thus, seeds of this study received none of the natural conditioning that might confound results if seeds were sown before weather conditions were favorable for germination.

The seedbed was divided into six blocks, each containing six rows. Each speciestreatment combination was assigned randomly to one row within each of the six blocks, and 40 seeds were sown per row. Thus, each species, soaked in hydrogen peroxide solution or in water, appeared in six rows, and there were 240 seeds of each species per treatment.

The seedbed was examined weekly for more than 2 months and intermittently thereafter into the following summer.

Results

Every examination revealed that seed germination and seedling survival were affected by the hydrogen peroxide treatment. Separate results are presented for each species.

Douglas-fir

Soaking in dilute hydrogen peroxide solution clearly increased and speeded field germination of Douglas-fir seed (table 1). Total germination of peroxide-soaked seed, 54.6 percent, was significantly greater (probability 99.6 percent) than the 30.5 percent for seed soaked in water only. Germination of peroxidetreated seed was complete within 7 weeks of sowing, compared with 9 weeks for the watersoaked seed. The influence on the start of germination was even more pronounced; after 4 weeks, 50.8 percent of peroxide-treated seed had germinated, but only one watersoaked seed had done so. All Douglas-fir germination occurred during the first season.

Early mortality of Douglas-fir seedlings from water-soaked seeds was more than from peroxide-soaked seeds, but by the end of the

TABLE 1.--Comparative total germination of water-soaked and hydrogen peroxide soaked seed

Species	Seed treatment	Cumulative germination after-- 3 weeks	7 weeks	10 weeks	15 weeks	1 year	1 year, 2 months
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Douglas-fir........	H_2O	0	27.0	30.5	30.5	30.5	30.5
	1% H_2O_2	34.0	54.6	54.6	54.6	54.6	54.6
Ponderosa pine.....	H_2O	0	11.7	31.2	33.8	45.0	45.0
	1% H_2O_2	0	1.2	20.4	27.5	47.1	47.5
Sugar pine.........	H_2O	0	.4	.8	3.8	33.8	34.2
	1% H_2O_2	0	.4	2.1	4.6	43.8	46.2

study the situation was reversed. After 15 weeks, 26.7 percent of the seedlings from peroxide-treated seeds and 41.0 percent of those from water-soaked seeds had died (table 2). By the end of the study, the equivalent values were 87.8 and 72.1 percent, respectively. The difference within each pair of values is significant; the probability is 97 percent. Though more than twice as many peroxide-treated seeds produced seedlings, mortality reduced the 1-year-old survivors to 16, 1 less seedling than from watersoaked seeds.

Ponderosa Pine

Treating ponderosa pine seed with hydrogen peroxide did not affect its total germination (table 1). By June of the second season, 45.0 percent of water-soaked and 47.5 percent of peroxide-soaked seed germinated. However, germination of peroxide-treated seed lagged consistently behind that of water-soaked seed during the first season. After 15 weeks, the difference in total germination between 33.8 and 27.5 percent for water- and peroxidesoaked seed, respectively, bears a 68-percent probability of being real. One-fourth of the final number of seedlings from water-soaked seed and more than two-fifths of those from peroxide-treated seed appeared in the second spring.

Cumulative mortality was lower for seedlings from peroxide-treated seed, with a 69percent probability that the difference was real. The early trend for lower mortality of seedlings from peroxide-treated seed endured for ponderosa pine into the second year. Seedling mortality in all ponderosa pine treatments was notably low.

Sugar Pine

Soaking sugar pine seed in hydrogen peroxide solution apparently slightly improved germination. The difference in total germination-34.2 percent for water-soaked seed and 46.2 percent for peroxide-soaked seed--bears a 90percent probability of being real. About onetenth of the total sugar pine germination appeared in the first season.

By the end of the study, cumulative mortality was similar for sugar pine seedlings originating from seed of either treatment. However, among the few seedlings present, the difference between 6.1-percent mortality for 15-week-old seedlings from water-soaked seed and 2.7 percent for seedlings from peroxide-soaked seed bears a 70-percent probability of being real. Mortality of sugar pine seedlings constituted only a minor part of total germination.

TABLE 2.--Mortality of seedlings originating from water-soaked or hydrogen peroxide soaked seed

Species	Seed treatment	Cumulative seedling mortality[1] after--		
		7 weeks	15 weeks	1 year
		Percent	*Percent*	*Percent*
Douglas-fir........	H_2O	0	41.0	72.1
	1% H_2O_2	6.1	26.7	87.8
Ponderosa pine.....	H_2O	0	5.6	6.5
	1% H_2O_2	1.8	2.6	3.5
Sugar pine.........	H_2O	0	6.1	11.0
	1% H_2O_2	0	2.7	12.6

[1] Based on final determination.

Discussion

Results of this study have created more questions than they have answered. Soaking seed in a 1-percent hydrogen peroxide solution may affect subsequent seedling germination and survival in the field. The hydrogen peroxide solution definitely elicits responses that vary by species.

Our primary query--whether hydrogen peroxide has merit for treating seed to be sown in the field or nursery--has not been fully answered. Clearly, germination of Douglas-fir seed can be speeded by treatment with hydrogen peroxide. Such treatment might replace the usual lengthy stratification when time is significant. Germination of ponderosa and sugar pine was not materially speeded; ponderosa pine germination may even have been delayed. Total germination of Douglas-fir and sugar pine possibly can be increased by this treatment.

Hydrogen peroxide treatment of the seed apparently had a generally beneficial effect on early seedling survival. For all three species, mortality of seedlings at 15 weeks was lower for peroxide-treated seeds than for water-soaked seeds. The statistical probability of the difference was 97 percent for Douglas-fir and 70 percent for ponderosa pine and sugar pine. Under conditions more favorable to Douglas-fir survival than in this study, a substantial gain in established seedlings might accrue in late sowings by use of peroxide-treated seed. Logically, the influence of the treatment should wane as seedlings develop secondary growth and are exposed to the various agents of mortality.

Growth of all surviving seedlings was normal, and there was no evidence of any lingering effect from hydrogen peroxide treatment.

The effect of hydrogen peroxide needs further investigation to clarify trends observed in this study--delayed germination of ponderosa pine, slight speeding of sugar pine germination, and reduced early seedling mortality of all three species. A range of peroxide concentrations and soaking periods should be tried on a number of seed lots of each species. Stronger solutions or longer soaking periods very likely will cause greater response by ponderosa and sugar pine.

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