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Overcoming Temperature-dependent Dormancy of Southwestern Ponderosa Pine Seed

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Germination of seeds *Pinus ponderosa* var. *scopulorum* is temperature-dependent. A laboratory experiment under fluctuating warm (16 hours at 24-27° C) and cold (8 hours at 3-4° C) temperatures showed that seeds given 48-hour aerated soaks of gibberellic acid (GA₃), thiamin, or nine other materials, including water, germinated faster than controls.

Keywords: Ponderosa pine, seed germination, dormancy

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Management Implications

Planting is the surest method of regenerating ponderosa pine in the Southwest, but it is very expensive. Natural regeneration is much less costly but is limited to sedimentary soils. On soils of volcanic origin, first-year seedlings are quite small because of late germination and a short growing season. These small trees are highly susceptible to frost heaving and drought during the fall and winter. The potential for direct seeding exists on thousands of acres of volcanic soils in the Southwest if seeds can be stimulated to produce a larger tree, better able to withstand the rigors of frost heaving and drought. In addition, rapid germination would likely mean that much less seed would be required than formerly used.

Germination of southwestern ponderosa pine seed is temperature-dependent (Pearson 1950, Larson 1961). Seeds will germinate in a few days under optimum temperatures (20-25° C) without pretreatment. Germination is slowed considerably, however, under the fluctuating temperatures of the southwestern United States, where the diurnal range in summer is commonly 4-27° C. Germination in the field does not begin until midsummer when minimum soil temperatures reach 13° C (Larson 1961). A summer rainy season begins around July first, with germination following in mid-July to early August. There are only 30-50 days after germination for the seedling to become established before the onset of freez-

ing nighttime temperatures in late August or early September. The size of seedling tops and roots is important in determining if the young tree will be able to withstand the drought and frost heaving of the first fall and winter (Schubert et al. 1970, Heidmann 1976). Because the growing season is short and seedling size is vital to initial survival, any measures which will speed up germination are important. In addition, seeds which germinate rapidly are exposed to predation by seed eating rodents for a shorter period of time.

Although seeds of many coniferous species require moist stratification or some other pretreatment for germination (Pharis and Kuo 1977, U.S. Department of Agriculture 1974), southwestern ponderosa pine does not. Seeds will germinate rapidly in a germinator without pretreatment. Aerated soaking in water or gibberellic acid (GA₃)² will produce 50% germination in a few days at 20-25° C.³ Thus, southwestern ponderosa pine seeds germinate rapidly at optimum temperatures. In the field, however, low night temperatures hinder germination.

Many means of hastening germination of conifer seeds have been tried with varying degrees of success (Lavender et al. 1964, McLemore 1971, Riffle and Springfield 1968, Trappe 1961, Biswas et al. 1972, Hall and Galsky 1973, McBride and Dickson 1972, Allen 1960, Barnett 1971). Because most of these experiments have been

²Abbreviations used: GA₃: gibberellic acid; NAD: nicotinamide adenine dinucleotide; ADP: adenosine-5-diphosphate; CGMP: guanosine-3',5'-cyclic monophosphate; CAMP: adenosine-3',5'-cyclic monophosphate; ATP: adenosine-5'-triphosphate; BA: N6-benzyladenine; ABA: abscisic acid.

³Data collected by L. J. Heidmann, on file at Research Work Unit, Flagstaff, Ariz., 1981.

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conducted in germinators or greenhouses at optimum temperatures, research is still needed to determine how to stimulate germination at field temperatures. For southwestern ponderosa pine, aerating seeds in gibberellic acid (GA₃) gave 50% germination in 5 days in fluctuating warm and cold temperatures, compared to 7 days for aeration in distilled water.³ This reports a study to screen various compounds for their ability to stimulate germination of southwestern ponderosa pine seeds in a fluctuating warm and cool environment. Successful procedures would ultimately be applied to seeds used in field sowing.

The Study

The study, which was begun in 1977, tested several compounds reported in the literature to hasten seed germination (table 1). Southwestern ponderosa pine (*Pinus ponderosa* var. *scopulorum*) seeds were collected on the Apache-Sitgreaves National Forest (elevation 2150 m) in 1971. After extraction, the seeds were soaked in water for 24 hours to separate sound from hollow seeds. Seeds were then dried at air temperature to below 10% moisture content and stored in plastic bags at -11° C. Seeds

used in the experiment had a moisture content of 6.3% and were soaked in a 30% solution of hydrogen peroxide (H₂O₂) for 20 minutes to sterilize the seed coats (Trappe 1961). Treatments, (table 1) were applied by aerating the seeds in 250 ml of solution for 48 hours at room temperature (20-24° C). Solutions of GA₃ were prepared by dissolving the material in 2 ml of ethanol and diluting to volume with distilled water. N6-benzyladenine (BA) was dissolved in 0.1 M HCl and diluted. All other compounds were dissolved in distilled water and diluted to volume. After treatment, seeds were removed from the solutions, rinsed with distilled water, and blotted dry on paper towels. Seeds were then placed in Petri dishes on Whatman number 5 filter paper which had been pre-wetted with 1 cc of distilled water. The experiment consisted of 3 randomized blocks of 26 treatments, each containing 50 seeds. Each replication was placed on a separate tray and then put into a refrigerator maintained at a temperature of 3-4° C. After 8 hours in the refrigerator the trays were removed and placed in a seed germinator for 16 hours under subdued light at a temperature which varied from 24° to 27° C. This procedure, which was repeated every day until conclusion of the study, was meant to simulate roughly the conditions encountered in the field. Each morning after onset of germination,

Table 1.—Effects of different treatments on germination of seeds of southwestern ponderosa pine

Treatments	Concentration	Days to 50% germination	Germination value	Total number of seeds germinating
GA ₃	1.0 mM	6.7 a ¹	27.32	125
GA ₃	0.1 mM	6.7 a	29.33	129
Thiamine	1.0 mM	6.0 a 6.7	30.71	130
Thiamine	0.1 mM	6.7 a	26.70	118
Cytochrome C	10 μM	7.0 ab	24.61	122
NAD	1.0 mM	7.0 ab	24.96	123
ADP	1.0 mM	7.0 ab	28.35	128
CGMP	0.1 mM	7.0 ab	25.88	126
Aeration in distilled water		7.3 ab	24.15	122
CAMP	0.1 mM	7.3 ab	25.74	121
NAD	0.1 mM	7.3 ab	20.33	121
ATP	0.1 mM	7.7 ab	23.20	121
Adenine	0.1 mM	7.7 ab	23.13	119
Tryptophan	1.0 mM	7.7 ab	25.92	130
Cytochrome C	1.0 μM	8.0 abc	22.20	123
Adenine	1.0 mM	8.0 abc	20.14	114
Thiourea	0.1 mM	8.0 abc	17.88	103
CAMP	1.0 mM	8.3 abc	19.67	114
ATP	1.0 mM	8.7 abc	18.93	111
Tryptophan	0.1 mM	8.7 abc	21.87	121
CGMP	10 μM	8.7 abc	20.91	118
ADP	0.1 mM	9.3 abc	17.76	110
Thiourea	1.0 mM	9.3 abc	19.31	111
BA	0.1 mM	9.7 bc	15.15	99
Control		10.3 c	21.01	129

¹Treatments followed by the same letter are not significantly different (P = 0.05).

seeds which had a radicle of 3 mm or longer were recorded as germinated and discarded. Filter paper was moistened daily as needed. The experiment was terminated at the end of 26 days. Days to 50% germination, total germination, and germination values (Czabator 1962) were analyzed by analysis of variance. The germination value (GV) is calculated as mean daily germination (number of full seed germinating at the end of the test divided by days to end of test) times the peak value (cumulative peak germination percent divided by days to peak.)

Results and Discussion

Southwestern ponderosa pine seeds aerated in several solutions germinated significantly faster than controls ($P=0.05$) (table 1). Seeds treated with 1.0 mM and 0.1 mM solutions of GA_3 and thiamin reached 50% germination in 6.67 days compared to 10.3 days for the control. This was not significantly better, however, than several other treatments, including aeration in water, which took 7.3 days for half of the seeds to germinate (table 1). There were no differences in germination value or in total numbers of seeds germinating (table 1). The analysis was originally run for all 26 treatments, but treatment with BA at 1.0 mM significantly repressed germination ($P=0.01$) and was omitted from further analysis.

It is not possible to pinpoint the cause of temperature-dependent dormancy of southwestern ponderosa pine on the basis of this experiment. There are some indications, however, of the mechanisms. Khan (1975) states that GA, cytokinin, and ABA play primary, permissive, and preventive roles in germination of seeds. Cytokinins oppose the actions of inhibitors but have little activity by themselves. Thus, germination will occur in the presence of ABA if cytokinin and GA are present, and in the absence of ABA if GA is present. Because germination in southwestern ponderosa pine seeds is rapid at temperatures over 20° C, we can assume that GA is present at these temperatures. ABA may also be present, in which case cytokinin is necessary for germination. As temperatures drop, the hormonal balance may change (Khan 1975). It is, therefore, possible that at low temperatures GA levels drop, while levels of cytokinin and ABA either rise or stay the same. The presence of both at low temperatures is indicated by the failure of BA to accelerate germination and the success of GA_3 .

It has been suggested that moving water removes inhibitors from the seeds which allows germination to proceed.⁴ It seems likely, however, that if inhibitors are leached from the seeds by moving water, cytokinins and GA would be also. A more likely explanation, because seeds aerated in water also germinated significantly faster than the control, is that oxygen plays a role in triggering germination.

Another possibility is that dormancy may be related to the amount of moisture imbibed by the seeds. Sukhorosova (1966), cited by Ovcharov (1969), found tem-

perature differences to be extremely important in germination of maize seeds. Under favorable temperature, seeds began to germinate even though they had absorbed only half of the required moisture. When temperatures were low, germination did not begin until swelling of the seed was complete. In the laboratory, southwestern ponderosa pine seeds germinate readily on barely moistened filter paper in a few days, but in the field germination may be delayed until two or three weeks after the first rains in early July. Summer rains in Arizona during July and August are usually sporadic and of short duration and intensity. Because the minimum temperature at night drops to a few degrees above freezing, it may be that the seeds need to imbibe the maximum amount of moisture before germination can begin. This might take several showers. Moisture alone, however, does not appear to be the controlling factor, since soaking seeds of southwestern ponderosa pine in water without aeration depressed germination (Larson and Schubert 1969).

If thiamin stimulates seed germination, its role is not clear. Thiamin is known to function as a coenzyme and as such may enhance the activity of other compounds in the metabolic pathway.

In order to determine if GA_3 , thiamin, and other substances act independently of oxygen in stimulating germination of southwestern ponderosa pine seeds, additional studies of a more sensitive nature should be conducted. These experiments should involve the use of carriers such as acetone so that materials can be introduced into the seed quickly, thus, eliminating the need for aeration (U.S. Department of Agriculture 1975, Tao and Khan 1974). It would also be beneficial to conduct experiments to identify promoters and inhibitors which may be present in seeds at both high and low temperatures.

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⁴Personal correspondence with Lavender, 1979.

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