# Effects of Distillery Wash Derivatives and Stratification on Germination of Italian Alder and Douglas-fir Seeds

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*The effects of distillery wash derivatives (DWD) and stratification on germination were studied for 2 species with different seed structure and dormancy: Italian alder (*Alnus cordata *Loisel) and Douglas-fir (*Pseudotsuga menziesii *(Mirb.) Franco). Seeds were subjected to 2 pretreatments before germination tests: (1) prechilling at 4 °C (39 °F) for 21 days or (2) no prechilling. Subsequently, germination tests were conducted in water (control) or DWD solutions (10 and 30 ppm). The use of DWD solutions (10 and 30 ppm ) for imbibition during germination test did not affect germination in Douglas-fir but did increase the speed and early germination percentage (third day) in Italian alder.* Tree Planters' Notes 48(3/4): 55–59; 1997.

Delayed germination of trees and shrubs seeds due to seed dormancy can be a serious problem in the nursery. Germination over a 1- to 3-year period represents a powerful biological strategy to aid natural propagation, but its occurrence in the nursery leads to irregular stocking. If the seeds do not germinate together, the final grade-out of the seedlings will greatly vary, because seedlings emerging early will tend to suppress the growth of those emerging later (Cullum and Gordon 1993). Increased speed of germination, thus avoiding uneven and erratic plant emergence, reduces the risk of fungal attacks and leads to the production of planting stock of more homogeneous size (Bonner and others 1974).

At present, there are commercially available natural and synthetic products containing amino acids, hormones, carbohydrates, enzymes, vitamins that stimulate many physiological processes and can be used to improve germination, growth, harvest, stress resistance, water, and nutrient uptake (Halmer 1989; Heydecker and Coolbear 1977; Oplinger and others 1978; Orsi and Tallarico 1983; Siviero 1993). A natural product that we have called DWD (distillery wash derivatives), which is not available for market, has improved germination rate in different species of agricultural seeds (Di Monte and others 1995). DWD is a light-brown, dense and viscous syrup, a by-product of sugar beet processing that contains amino acids, betaine, glycerol, various fatty acids, esters, and unfermented sugars. Because of its composition and positive effects on agricultural seeds, this product could also improve tree seed germination. The aim of this paper is to study the effects of DWD on seed germination in Italian alder (*Alnus cordata* Loisel) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco).

These 2 species show differences in seed structure and the cold requirements for germination. Seeds of Italian alder are small nuts, 350,000 to 550,000/kg (160,000 to 250,000/lb) (Piotto 1992), containing no endosperm and with a thin seedcoat in comparison to that of Douglas-fir. Seed viability is generally low (30 to 60%) and dormancy is shallow, for ISTA (1993) rules do not prescribe prechilling before germination tests. No germination inhibitors have been found in seedcoats of Italian alder. When the seedcoats are removed, germination is rapid and complete; their mechanical resistance seems to cause dormancy (Rinallo 1979). Italian alder is often sown at the end of winter, to benefit from natural provided cold-moist conditions, or in spring after a prechilling for 3 to 4 weeks.

Seeds of Douglas-fir number 70,000 to 95,000/kg (32,000 to 434,000/lb), have a thicker seedcoat, contain endosperm, often show a germination percentage over 80%, and exhibit a type of dormancy that is broken by cold–moist pretreatments. Prechilling improves germination rate and germination percentage (Owston and Stein 1974), but marked differences can be observed between seedlots. For this reason, ISTA (1993) rules prescribe double testing (prechilling and no prechilling) before germination of Douglas-fir. Dormancy depends on seedcoat inhibition and immature embryos, with extreme variablity because some seedlots are able to germinate promptly without any pretreatment (Vanesse 1974; Gordon 1979). Most nurseries first soak Douglas-fir seed in tapwater and then cold treat for 3 to 8 weeks before spring sowing.

1978; De Matos Malavasi and others 1985). The germinated seeds were counted every 2 or 3 days until the 28th day to calculate the germination percentage (G%) on the 3rd, 7th, and 28th day (final germination) and the mean time to complete germination (MTG) (Bewley and Black 1986). MTG is a measure of the speed of germination:

$$\Sigma (t \times n) / \Sigma n$$

where:
$t$ = the time in days, starting from the day of the beginning of the test
$n$ = the number of seeds completing germination on day $t$

Quicker germination is associated with lower MTG values ( Bewley and Black 1986).

Statistical differences were estimated on the basis of the analysis of variance (Gomez and Gomez 1984) of MTG's and G% at arcsin $\sqrt{}$% (tabulated values are the untransformed data). Comparison of means was performed following the Duncan's multiple range test (Harter 1960) at $P < 0.01$.

## Results and Discussion

Data presented in tables 2 and 3 show that statistically significant differences were only attributable to main factors. In Italian alder, MTG was more influenced than percentage germination, with this effect being more noticeable at the beginning than at the end of the germination process. Rapid germination processes are often associated with remarkable early G%'s. In Douglas-fir, both cold treatment and DWD treatment affected speed and total germination.

**Pregermination treatments (factor A).** In both species, seeds that were not subjected to prechilling (A2) showed a low G% at the beginning of the trial (3rd day in Italian alder and 7th day in Douglas-fir). Final germination (28th day) of pretreated Italian alder seeds did not significantly differ from those that were stratified (A1). In Douglas-fir, cold–moist pretreatment resulted in higher early and final germination of prechilled seeds in comparison to untreated seeds (table 2).

Germination speed, expressed as MTG, was always improved by stratification (table 2). On the 3rd day, G% was equal to almost 70% of final germination percentage in stratified Italian alder seeds (A1), whereas no germination could be observed in untreated seeds. Such an effect was expected in both species because it shows consistency with widespread nursery practice in which both Italian alder or Douglas-fir seeds are subject to stratification before spring sowing (Gordon and Rowe 1982). Although this trial was conducted with only 1 provenance, the results suggest a probable need of modification of ISTA rules, that prescribe prechilling before the germination test for Italian alder.

**DWD concentration during germination test (factor B).** The use of DWD solutions during germination did not affect the rate or the completeness of germination in Douglas-fir but it did accelerate the germination process in Italian alder (table 3). Furthermore, DWD solutions increased early G% (3rd day), and thus germination speed, in the latter species. There was no difference between 10 and 30 ppm (table 3). It was hypothesized that the combination of sucrose and amino acids present in DWD might have resulted in rapid embryo development in Italian alder. Douglas-fir seeds were not affected by the DWD concentrations tested, probably due to their thicker seedcoat.

## Conclusions

Results of the study have indicated that:

- The use of DWD solutions (10 and 30 ppm) during germination tests accelerated the germination process in Italian alder but did not affect the rate or the completeness of germination in Douglas-fir. We hypothesize that differences in seed coat structure could explain different behaviours and responses.
- Stratification strongly influenced both early G% and MTG in Italian alder and early, final G% and MTG in Douglas-fir. Although prechilling is prescribed by ISTA (1993) rules for germination tests in the former species it is not indicated for Italian alder. These results could suggest a probable need of modification of ISTA rules.

Although preliminary in nature, the results show that DWD improved tree seed germination in 1 species. Further research is required on a wider range of species, as DWD is cheap, safe, and easy to use.

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## Materials and Methods

Italian alder and Douglas-fir seeds were collected, respectively, in 1991 in Tivoli, Rome (41,58 N; 12,48 E; 50 m asl) and in 1980 in Albany, Oregon (44,38 N; 123,06 W; 700 m asl). They were stored at −3 °C (27 °F).

For Italian alder, germination percentage was 48%; purity, 89%; moisture content, 7%; and the number of cleaned seeds per kilogram, 540,000. For Douglas-fir, germination percentage was 84.5%; moisture content, 8.5%; and the number of cleaned seeds per kilogram, 88,500. Seeds for trials were obtained from four 50-g (1.7-oz) samples withdrawn at random from both seedlots.

The DWD composition was sucrose, 50%; betaine, 20%; valine, 10%; isoleucine, 5%; leucine, 3%; tyrosine, 10%; glycine, glutamic acid, and serine, trace; other components, 2%. For each species, a 2-factor split-plot (Gomez and Gomez 1984) design with 4 replications was used in the experiment. There were 2 pregermination treatments; (A1) prechilling at 4 °C (39 °F) for 21 days and (A2) no prechilling. Three concentrations of DWD were tested: (B1) Control (0 ppm); (B2) 10 ppm; (B3) 30 ppm. The 6 treatments resulting from treatment combinations are shown in table 1.

During the chilling period, seeds were placed in germination boxes with water. They were then rinsed in water and put in new boxes containing DWD solutions at different concentration (0, 10, or 30 ppm) for the germination test. Unchilled seeds were soaked in water for 24 hours at room temperature and then placed in germination boxes containing DWD solutions (0, 10 or 30 ppm). Prechilling was performed so that all seeds could begin germination tests at the same time in a cabinet germinator. In both species, four 50-seed replicates were prepared for each of the 6 experimental treatments (table 1). Seeds were placed on top of filter paper in covered germination boxes (diameter = 16 cm) in which water or solutions uptake was easily allowed through strips of filter paper from a reservoir at the bottom (figure 1A&B). Germination boxes contained 250 ml of water or DWD solutions, depending on experimental treatment.

Germination conditions were those prescribed by the ISTA (1993) rules: 8 hours at 30 °C (86 °F) in light plus 16 hours at 20 °C (68 °F) in dark. Experimental treatments A2B1 for Italian alder and A1B1 and A2B1 for Douglas-fir are to be considered under the standard conditions as they followed ISTA (1993) recommended pregermination treatments: no prechilling for Italian alder, double test (prechilling and no prechilling) for these 2 species as already stated. Germination was recorded when the radicle length exceeded 2 mm (Danielson and Tanaka

**Table 1**—*Prechilling (factor A) and distillary wash derivative (DWD) (factor B) treatments applied to Italian alder and Douglas-fir seeds*

| Pregermination treatment | DWD conc. (ppm) |
|---|---|
| Prechilling (A1) | 0 —control (B1)<br>10 (B2)<br>30 (B3) |
| No prechilling (A2) | 0—control (B1)<br>10 (B2)<br>30 (B3) |

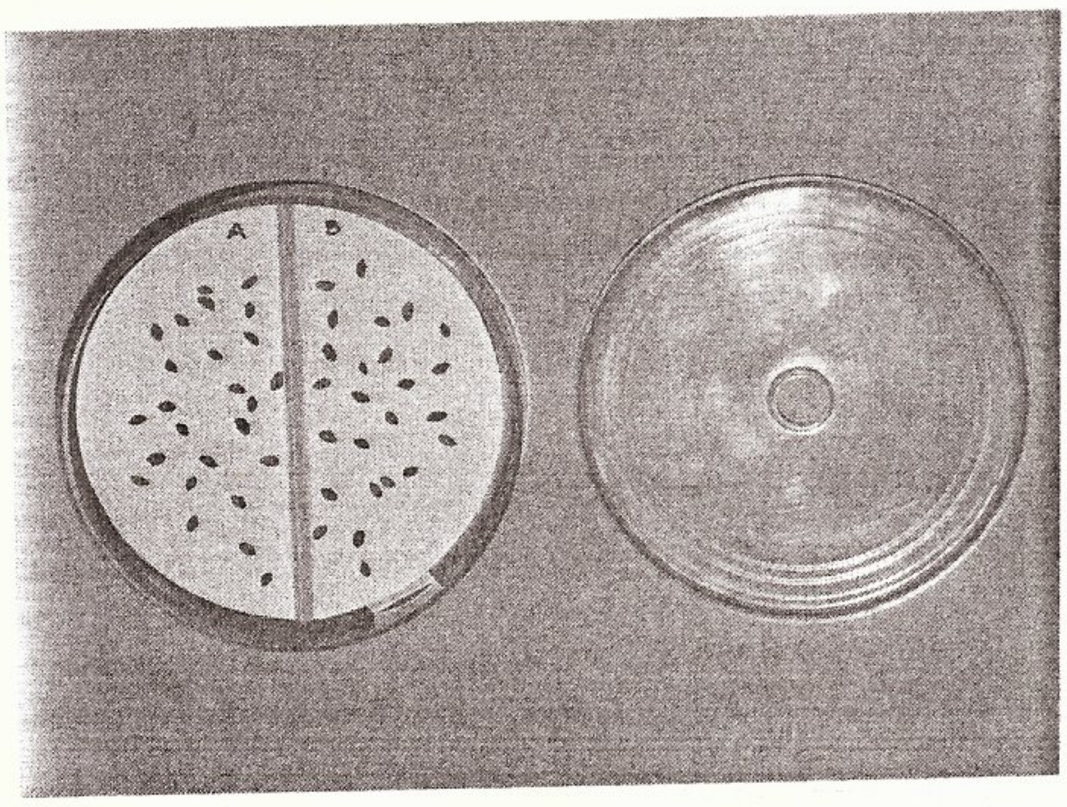


**Figure 1**—*Germination box, seen from the top (A); detail of the b showing uptake water or solutions system (B).*

**Table 2**—*Germination percentages on the 3rd, 7th, and 28th days and mean time to complete germination (MTG) of seeds of Italian alder and Douglas-fir subjected to different pregermination treatments*

| | Italian alder | | Douglas-fir | |
|---|---|---|---|---|
| | Prechilling | No prechilling | Prechilling | No prechilling |
| Germination (%) | | | | |
| 3rd day | 35.0 B b | 0.01 A a | ng | ng |
| 7th day | 46.9 A a | 51.9 A a | 35.9 B b | 4.2 A a |
| 28th day | 48.2 A a | 57.0 A b | 73.5 B b | 67.5 A a |
| MTG (days) | 3.9 A | 6.3 B | 9.2 A a | 14.8 B b |

**Note:** ng = no germination observed. Values are averages for 3 distillery wash derivative (DWD) solutions. For each species, means within a row followed by different capital letters are significantly different at $P < 0.01$ according to the Duncan's multiple range test (Harter 1960); means within a row followed by different lowercase letters are significantly different at $P < 0.05$.

**Table 3**—*Germination percentages on the 3rd, 7th, and 28th day and mean time to complete germination (MTG) of seeds of Italian alder and Douglas-fir imbibed with distillery wash derivative (DWD) solutions of 0, 10, & 30 ppm during the germination test*

| | Italian alder | | | Douglas-fir | | |
|---|---|---|---|---|---|---|
| | 0 ppm | 10 ppm | 30 ppm | 0 ppm | 10 ppm | 30 ppm |
| Germination (%) | | | | | | |
| 3rd day | 6.9 A a | 12.8 B b | 10.7 AB b | ng | ng | ng |
| 7th day | 52.6 A a | 49.3 A a | 47.8 A a | 16.7 A a | 14.6 A a | 19.7 A a |
| 28th day | 56.3 A a | 52.4 A a | 49.3 A a | 66.6 A a | 70.7 A a | 75.0 A a |
| MTG (days) | 5.6 b B b | 4.9 AB a | 4.8 A a | 12.4 A b | 12.0 A ab | 11.5 A a |

**Note:** ng = no germination observed. Values are averages for 2 pregermination treatments. For each species, means within a row followed by different capital letters are significantly different at $P < 0.01$ according to the Duncan's multiple range test (Harter 1960); means within a row followed by different small letters are significantly different at $P < 0.05$.

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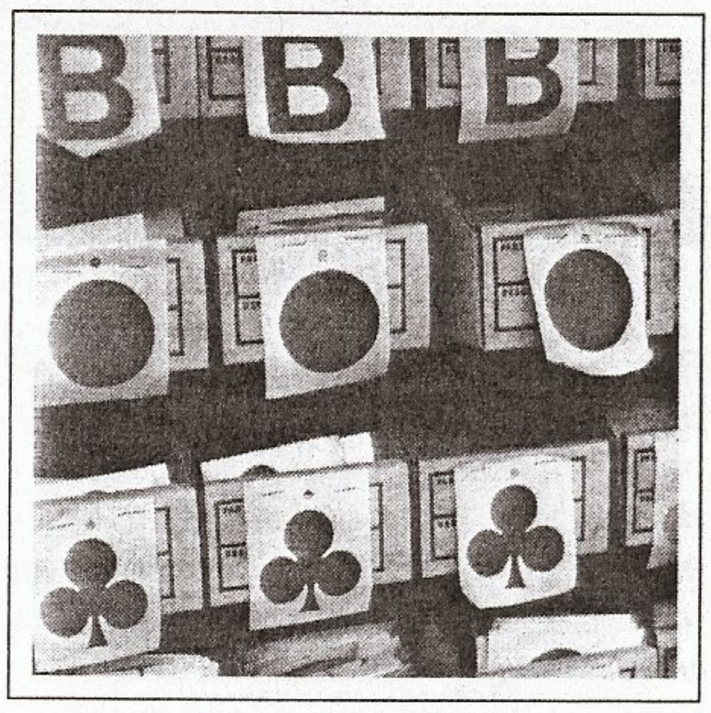

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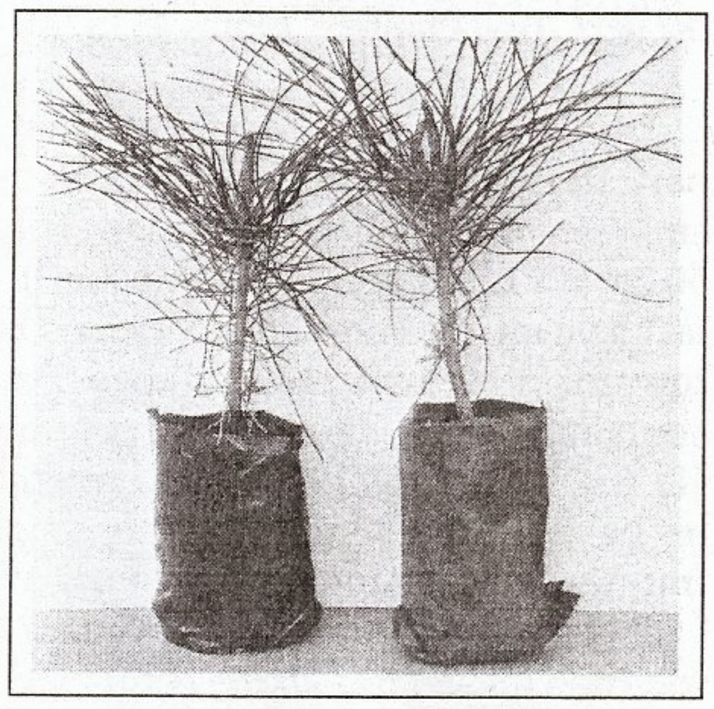

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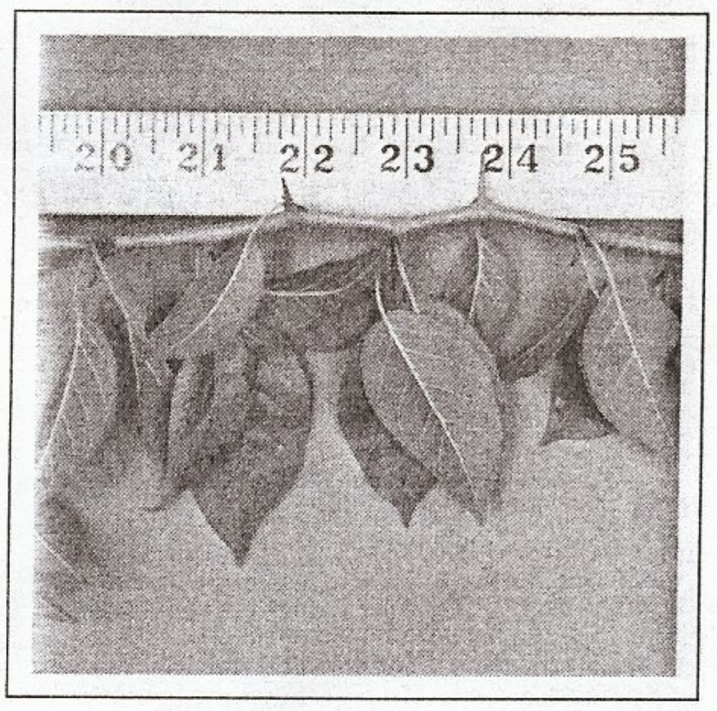


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