Marking Tree Seeds with Spray Paint for Germination Studies

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ABSTRACT: *I evaluated the potential use of spray paint for marking conifer seeds for germination studies in forest nurseries. For bulk seedlots of large-seeded species like western white pine* (Pinus monticola), *ponderosa pine* (Pinus ponderosa), *and Douglas-fir* (Pseudotsuga menziesii), *paint had little or no effect on six different germination parameters, but negatively affected germination of western larch* (Larix occidentalis). *On a family level with pine seeds, spray paint may or may not be appropriate depending on the specific objectives of the researcher and the level of conservatism used. West. J. Appl. For. 18(3):175–178.*
Key Words: Western white pine, ponderosa pine, Douglas-fir, western larch.

The ability to identify seeds and subsequent seedlings is often necessary in forest nursery seedling research. When a nursery parameter, such as time of seedling emergence (germination energy), is important, a simple technique like placing a colored plastic ring around the base of a seedling to differentiate it works well (Mexal and Fisher 1987). However, when specific families of seedlings need to be sampled in a nursery production setting, and seeds of those families are routinely combined to form a bulk seedlot, tracking the identity of subsequent seedlings is more difficult.

In one such study, El-Kassaby and Thomson (1996) maintained seed identity in a container nursery by dividing individual container cavities into three separate areas using a three-pronged divider, and hand-planting known families into each area based on random numbers. However, I was interested in looking at the effects of all nursery impacts (including planting with a precision seeder) on representation of families when sown as a bulk seedlot. I therefore needed a method of marking seeds by family so identities could be made after seeds passed through the seeder. In a preliminary study, after mechanical seeding, but before covering seeds with a grit top-dressing, painted seeds were easily identified and their exact locations mapped as they lay on the medium within individual container cavities. Because of epigeal germination in conifers, emerging seedlings were also identified as their seed coats were lifted through the top-dressing by the elongating hypocotyl.

Believing that a fine coating of spray paint would allow me to identify seeds *in situ* as mechanically planted and after germination, I conducted three experiments to determine the impact of spray paint on seed germination. For each experiment (which contained up to four species), appropriate seeds were soaked 48 hr in running water and naked stratified in cold (2 to 3°C) moist conditions for a species-dependent duration: western white pine (*Pinus monticola*) 120 days; ponderosa pine (*P. ponderosa*) 50 days; Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) 28 days; and western larch (*Larix occidentalis*) 28 days. After stratification, seeds were allowed to surface dry at 21°C. Seeds in paint treatments were spread into a one-seed-thick layer and painted. Paints were products of either Plasti-kote Co., Inc. (Medina, OH) or the Krylon Division of Sherwin-Williams Co. (Solon, OH) and contained various proprietary mixtures of propane, xylene, and ketones; none of the manufacturer labels provided detailed ingredient lists. Painted seeds dried rapidly, and were immediately hand-planted on a 1:1 (v:v) sphagnum peat moss:vermiculite medium within containers used for growing reforestation seedlings, then covered with a 5-mm-deep layer of silica grit, and placed within a production crop at the University of Idaho Forest Research Nursery greenhouses during March and April. Containers were irrigated to field capacity as soon as they were placed into the greenhouse. Daytime temperatures ranged from 21 to 29°C with nighttime temperatures of 18 to 21°C. I counted germination (defined as when the elongating hypocotyl was visible above the grit layer) daily for 35 days after planting. In Experiment 1, I evaluated germination one way, but in experiments 2 and 3, I evaluated germination six ways (Table 1). Germination parameters were compared for each species, but not among species.

NOTE: Kas Dumroese can be reached at Phone: (208) 883-2324; Fax: (208) 883-2318; E-mail: kdumroese@fs.fed.us. The author thanks G.E. Rehfeldt for providing the ponderosa pine families; G. Franc for assistance with collecting the western white pine families; M.F. Mahalovich for selecting western white pine families at the Coeur d'Alene nursery and to A. Eramian and the nursery crew for harvesting and processing the seeds; the Inland Empire Tree Improvement Cooperative for their generous funding; and D.L. Wenny, S.J. Morrison, and K.E. Quick of the University of Idaho Forest Research Nursery for gracious financial support and technical assistance. He is particularly indebted and thankful to R. Wallace for his invaluable statistical expertise. This article was written by a U.S. Government employee and is therefore in the public domain.

Table 1. Definitions of seed germination parameters used to evaluate spray paint.

Germination parameter	Description
Germination capacity (cumulative germination; total germination) *(GC)*	The percentage of seeds that germinate during the test time. A measure of germination completeness.
Peak value *(PV)*	A measure of germination speed. *PV* is the maximum value obtained using: $PV = DCG \div \text{days since start of test}$ where *DCG* is daily cumulative percent germination (Czabator 1962).
Germination value *(GV)*	*GV* combines germination speed and completeness: $GV = (GC \div D) \bullet PV$ where *D* equals the number of days in the test (Czabator 1962).
Germination value prime *(GV')*	A refinement of Czabator's (1962) GV: $GV' = (\Sigma PV \div N) \bullet GC \bullet 10$ where *N* equals the number of observations used to determine *PV* (Djavanshir and Pourbeik 1976).
Germination rate (GR_{50})	The number of days required for 50% of the seeds to germinate (Ching 1959).
Germination rate prime (GR'_{50})	The number of days required for 50% of the germinating seeds to germinate (Thomson and El-Kassaby 1993).

Experiment 1

My objective in Experiment 1 was to determine if orientation of the painted side of the seed (either toward substrate or away from substrate) affected germination. Western white pine seeds were divided into three treatments (control, no paint; painted side placed against medium; painted side directed away from medium). Treated seeds were sprayed with one color of paint. I replicated each treatment ten times with 20 seeds per replicate. I used PROC ANOVA in Statistical Analysis Software (SAS 1989) to compare treatments. Residuals were plotted and their distribution was normal, independent, and homogeneous, making data transformation unnecessary. Alpha level was 0.05. Germination capacity was unaffected by treatment ($P = 0.7$), replication ($P = 0.8$), or the interaction ($P = 0.6$). Mean germination (± 95% confidence interval) of the control, paint oriented toward the substrate, and paint oriented away from the substrate was 76% ± 6%, 79% ± 4%, and 77% ± 4%, respectively.

Experiment 2

The objective of Experiment 2 was to see if different species were affected by painting. For all four species, eight colors of paint and a single nonpainted control (nine treatments total) were evaluated for effects on germination. Seeds were painted and planted as described above with the painted side directed away from medium. Each treatment was replicated three times with 20 seeds per replicate. I compared data using logistic regression (the dependent variable was total germination achieved with each paint color to total germination of the control) and the Kruskal-Wallis Test (for nonparametric analysis of variance models comparing the ranked painted value for all germination parameters with those for the control) in SAS (SAS 1989). For western white pine, ponderosa pine, and Douglas-fir, germination parameters were unaffected by treatment when alpha = 0.05 (Table 2). However, GR'_{50}, germination energy when expressed as the number of days required for 50% of the germinating seeds to germinate, was borderline for ponderosa pine with treated seeds germinating about one day slower than controls.

Paint significantly reduced most germination parameters for western larch, indicating possible phytotoxic effects. Unfortunately, proprietary issues and poor ingredient lists make meaningful discussion about which chemicals are involved difficult. In one brand, propane, listed as the sole ingredient, yielded significant effects on germination in one color but not in another; similar results occurred in the other brand for two colors containing ketones and xylene (Table 3). Logistic analysis indicated cumulative germination, when each paint was compared to the control, was similar for both pine species and Douglas-fir, but six of the eight colors significantly reduced germination capacity of western larch (Table 3).

Experiment 3

In Experiment 3, my objective was to see if paint had any deleterious effects on germination of specific families within a species. I used 20 families (seeds collected from individual trees) of western white pine and 14 families of ponderosa pine. Seeds were divided into two treatments [control, no paint; painted (one color per family)] then painted and planted as described above. Each treatment was replicated eight times with 20 seeds per replicate. I compared data using the nonparametric Wilcoxon Rank Sum Test (comparing the ranked painted value for all germination parameters with those for the control) in SAS (SAS 1989). For western white

Table 2. Experiment 2 means, 95% confidence intervals, and *P* values after analysis with the Kruskal-Wallis test.

Germination parameter	Treatment	Western white pine	Ponderosa pine	Douglas-fir	Western larch
GC	Control	82.0 ± 19.0	90.0 ± 12.4	90.0 ± 24.8	81.3 ± 15.2
	Paint	86.9 ± 18.1	90.0 ± 15.6	91.0 ± 9.6	69.0 ± 15
	P value	0.808	0.215	0.170	0.140
PV	Control	4.4 ± 0.5	5.5 ± 1.5	4.3 ± 1.5	3.3 ± 0.7
	Paint	5.2 ± 1.4	5.2 ± 0.8	4.0 ± 0.9	2.5 ± 0.7
	P value	0.162	0.307	0.302	0.038
GV	Control	10.4 ± 3.6	14.0 ± 5.0	11.3 ± 6.9	7.7 ± 3.0
	Paint	13.1 ± 5.5	13.4 ± 3.9	10.4 ± 2.8	5.0 ± 2.8
	P value	0.354	0.860	0.226	0.056
GV'	Control	18.2 ± 8.2	25.0 ± 10.0	19.5 ± 13.4	14.0 ± 8.0
	Paint	21.4 ± 9.0	23.7 ± 8.9	17.9 ± 5.0	9.4 ± 5.1
	P value	0.721	0.899	0.386	0.041
GR_{50}	Control	13.3 ± 1.4	12.3 ± 3.8	16.3 ± 2.9	17.7 ± 5.2
	Paint	13.4 ± 4.7	13.2 ± 1.6	17.1 ± 2.2	23.1 ± 8.4
	P value	0.566	0.115	0.706	0.046
GR'_{50}	Control	13.0 ± 0.0	12.0 ± 2.5	16.0 ± 2.5	16.7 ± 1.5
	Paint	13.2 ± 6.4	13.0 ± 1.6	16.6 ± 2.2	17.8 ± 3.3
	P value	0.786	0.052	0.423	0.184

Table 3. Experiment 2 *P* values using logistic regression comparing each color of paint to the control (no paint) for cumulative germination (CG).

Paint manufacturer and color	Listed ingredients	Western white pine	Ponderosa pine	Douglas-fir	Western larch
Plasti-kote Co, Inc, Medina, Ohio					
Easy Way Flat White (E-24)	Propane	0.196	0.752	1.000	0.076
Easy Way Red Primer (E-19)	Propane, xylene	0.196	1.000	0.752	0.043
Easy Way Shamrock Green (E-12)	Propane	0.625	0.571	0.571	0.023
Rust Not Enamel Brite Yellow (376)	Propane, xylene	0.310	0.308	0.752	0.002
Rust Not Enamel Baby Blue (383)	Propane, xylene	0.455	0.086	0.512	0.032
Krylon Division of Sherwin-Williams Co, Solon, Ohio					
Krylon Interior/Exterior Regal Blue (1901)	Ketones, xylene	0.810	0.411	0.287	0.003
Krylon Fluorescent Orange	Heptane, hexane, toluene, isobutane, propane, VM&P naphtha	0.310	0.769	0.163	0.001
Krylon Interior/Exterior Cherry Red (2101)	Ketones, xylene	0.810	0.308	0.512	0.322

pine, I had 6 different Wilcoxon tests for each of the 18 families for a total of 108 Wilcoxon tests. Using the Bonferroni adjustment for an alpha level of 0.05, my adjusted alpha (alpha') was 0.000462 (0.05/108) and no tests were significant. Using the unadjusted alpha level of 0.05, however, two-thirds of the families lacked any significant differences, but for six families at least one germination parameter was significant (Table 4).

Over the years, many germination parameters have been reported in the literature. In forest nurseries, however, the two most important germination parameters are probably cumulative germination (CG) and the speed to which 50% of the germinating seeds germinate (GR'_{50}) (Thomson and El-Kassaby 1993). Several of the other parameters (PV, GV, GV') are unitless numbers, making it difficult to determine if significant differences are really biologically important. That is why CG and GR'_{50} are better indices since their values are directly comparable and in units that nursery managers can easily recognize and compare. At the unadjusted level, GR'_{50} for western white pine was unaffected by painting, CG was increased in two families but decreased in one, and one family responded poorly to painting in five of the six germination parameters (Table 4).

For ponderosa pine, I also had 6 different Wilcoxon tests but only 14 families for a total of 84 Wilcoxon tests, making alpha' = 0.000595. Like western white pine, no tests were significant using the Bonferroni adjustment for ponderosa pine. Using the unadjusted alpha level of 0.05, however, only 53% of the families lacked significant differences for one or more germination parameters at 0.05. In two families, CG was affected by paint; it decreased in one family but increased in the other (Table 4). Unlike the generally slower germinating western white pine seeds, the faster germinating seeds of ponderosa pine resulted in 35% of all families (six affected) having a GR'_{50} value significantly altered by paint; however, these differences only ranged from 1.0 to 1.25 days, which

Table 4. In Experiment 3, germination parameters significantly affected by painting (+ = better than control; – = poorer than control; *P* value in parentheses) for specific families of either western white pine or ponderosa pine when tested with the nonparametric Wilcoxon Rank Sum Test without the Bonferroni adjustment.

Species and family	*CG*	*PV*	*GV*	*GV'*	GR_{50}	GR'_{50}
Western white pine						
4974	+ (0.039)					
4975					– (0.040)	
4976	– (0.010)	– (0.015)	– (0.013)	– (0.036)	– (0.018)	
4977		– (0.005)	– (0.010)			
4978	+ (0.016)		+ (0.013)	+ (0.027)		
5081		– (0.015)				
Ponderosa pine						
1	+ (0.042)	+ (0.020)	+ (0.021)	+ (0.021)		
4					– (0.008)	– (0.008)
5					– (0.032)	– (0.040)
9	– (0.019)	– (0.021)	– (0.021)	– (0.021)	– (0.015)	– (0.011)
10				+ (0.021)	+ (0.008)	+ (0.008)
14		+ (0.020)	+ (0.020)	+ (0.020)	+ (0.040)	+ (0.040)
15			+ (0.020)			
16				– (0.043)	– (0.011)	– (0.011)

may or may not be biologically significant or important to the nursery manager. For four families, the GR'_{50} value was poorer with paint while in two families the value after painting was better than the control.

Conclusions

Spray painting seeds of bulk seedlots of four species of conifers yielded varied results. If researcher objectives are to compare nursery responses using bulk seedlots, however, my results suggest that painting may be a viable method for marking seeds of western white pine, ponderosa pine, and Douglas-fir because germination, expressed six different ways, was unaffected. Western larch, however, showed an apparent phytotoxic response. Because of the poor listing of ingredients by manufacturers, and the possibility for phytotoxic responses, it may be prudent to do a preliminary germination test on seeds to be marked.

Similarly, if the objectives are to compare a cross-section of potential family responses within a seedlot, and potential differences among families are more important than the actual inherent differences of each family, painting may still be a viable method for marking seeds. In other words, if a variety of CGs and GR'_{50}s are known to exist among families, and a researcher wants to exploit those differences to access some aspect of nursery culture, then painting to identify families may be appropriate even though one or two families might yield different values (either positively or negatively). Appreciable variability still occurs but perhaps not accurately for specific families. However, if specific information is needed on each family, perhaps over multiple growing seasons, then although I found no significant responses with Bonferroni adjustments, researchers may find the differences observed in CG and GR'_{50} at an unadjusted alpha of 0.05 may limit the usefulness of this technique.

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