Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

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How long should we soak Ponderosa pine seed?

The current soak duration for ponderosa pine (*Pinus ponderosa* P. Laws. ex C. Laws.) [**Py**] of 24 hours was questioned. A relatively low value of 27.8 ± 0.5% moisture content was estimated from Quality Assurance testing for 84 – Py sowing requests and this value fell below the average of 31.7% for all species (see 'Stratification Moisture Content' in Volume 12 #1 of this Newsletter). Due to their large seed size and thick seed coat it was hypothesized that **Py may not be achieving optimal hydration in 24 hours and a longer soak may be required for this species**.

To investigate moisture content in Py after soaking it was decided to assess moisture content under lab conditions and under operational seed preparation conditions. The methods and results will be presented separately for these two investigations followed by a common discussion.

Materials and Methods

Lab Testing

A total of ten seedlots were selected representing a variety of sources and seed qualities (germination capacity [GC] ranging from 78 to 92%) (Table 1). For each seedlot a total of eight replicates of 100 seeds were counted and weighed to obtain the weight before soaking. Four replicates, of each seedlot, were soaked for 24 hours and four replicates were soaked for 48 hours. After soaking the replicates were again weighed (without surface drying) before placing seeds into germination dishes[1] for 28-days stratification at 2°C. After stratification was completed the seed was again weighed and the germination dishes transferred to a germinator set at 30°C for eight hours with lights and 20°C for 16 hours in the dark. Seed was considered germinated once the radicle was 4X the length of the seed coat. Germination capacity was assessed after 21 days.

Table 1[2]. Seed sources of Ponderosa pine (Py) used to evaluate moisture uptake.

Seedlot	Location	Coll Yr	Lat.	Long.	Elev.	SPZ	GC
3001	OK Falls	1976	49 00	119 00	579	TOD	91
5747	OatScott	1988	49 58	118 00	800	WK	80
5752	Arrow Pk	1988	50 07	117 53	610	WK	88
31257	Premier Lk	1991	49 57	115 42	850	EK	92
31825	Kerr Cr	1991	49 03	119 15	825	TOD	78
31845	West Kettle	1992	49 05	118 58	820	TOD	86
31906	D'Arcy	1991	50 33	122 29	380	SM	89
32000	Camoo Jct	1989	50 50	122 07	480	TOA	89
39301	Hat Cr	1993	50 48	121 34	900	TOA	85
42569	Clapperton Cr	1991	50 12	120 40	870	TOA	83

[1] Germination dishes consist of one piece of kimpack (22-ply wadding paper) , 50 ml of water and one filter paper onto which the seeds are placed.
[2] Coll Yr = Collection year; Lat.= latitude; Long. = longitude; Elev.= elevation in m.; SPZ= seed planning zone and GC= germination capacity (%)

Estimates of moisture content were obtained non-destructively by knowing the storage moisture content of each seedlot and the fresh weight before and after soaking and after stratification. The first step was to determine the oven-dry weight of each replicate by solving the following:

(1) Oven-dry weight = fresh weight * (1-moisture content)

Once the oven-dry weight is obtained one can calculate the moisture content at any other fresh weight using this formula:

$$\textbf{(2)}\quad \textbf{Moisture content} = \frac{\textbf{fresh weight} - \textbf{oven-dry weight}}{\textbf{fresh weight}}$$

To present the results as a percentage multiply by 100.

Seed Preparation Testing

Four of the seedlots in Table 1 had larger quantities of seed available for testing (**3001**-1400 g; **5747** – 300 g; **5752** – 120 g and **32000**- 1400 g). The average sowing request size for Py is 1941 grams since 1995, but can range from 29 to 3000 grams. This larger scale testing was performed to determine the 'operational' impact of the treatments. The high value and scarcity of Py seed limited the size and number of seedlots that could be used in this part of the trial. The amount of seed indicated above was divided into four fractions, weighed and two fractions were soaked for 24 hours and the other two for 48 hours. Within each soak duration one fraction was surface dried and the other fraction was not surface dried. The four fractions therefore represent a simple factorial experiment (without replication) looking at the effect of soak duration and surface drying. Each fraction was weighed after soaking (with or without surface drying) and then put in plastic bags and placed into cold stratification at 2°C for 28 days. Seed was weighed again after stratification was complete. This allowed for an estimate of the moisture content of the entire 'request'. Seed was then sampled and germination testing conducted the same as in Lab testing, outlined above.

Results

Lab Testing

The average moisture content of all ten seedlots was estimated at 35.2% after a 24-hour soak and 36.8% after a 48-hour soak. The two treatment became more similar during stratification and differed by only 0.4% at the end of stratification. Moisture content differences were not statistically significant between the 24- and 48-hour soak after imbibition or after stratification. From Analysis of Variance, seedlots were a statistically significant source of variation in moisture content after imbibition and after stratification. For example, variation between seedlots ranged from 32.4% to 39.9% following the 24-hour soak.

Differences in GC were slight (not statistically significant) between the two treatments with the 24-hour soak (79.6%) having slightly higher germination than the 48-hour soak (78.3%). Seedlots were a statistically significant source of variation for GC. The response did vary by seedlot as only half of the seedlots had a higher GC with the 24-hour soak. In three seedlots, falldowns of 8, 9 and 10% were experienced by extending the soak to 48 hours.

Seed Preparation

The four seedlots all followed the same pattern for moisture content progressing from 24-hour soak + surface dry (**28.9%**); to 24-hour soak (**30.7%**); to 48-hour soak + surface dry **(31.8%)**; and finally the 48-hour soak **(33.9**%). This is the pattern one would logically expect for moisture content. During stratification the surface dry samples gained a small amount of moisture (0.3%), but the non-surface dry samples lost about 0.6% moisture.

The four treatments all produced relatively good germination ranging from 82 to 86%. The 48-hour no surface drying treatment produced the highest GC (86%); followed by the 48-hour soak + surface dry (85%); 24-hour + surface dry (84%) and the 24-hour non-surface dried treatment (82%). Analysis of Variance indicated that the effect of surface drying was not statistically significant, while seedlot and soak duration were statistically significant sources of variation. All of the interaction terms were not statistically significant[3].

Discussion

The results do not indicate that a substantial consistent gain can be achieved by extending the soak duration in Ponderosa pine to 48 hours. In lab testing the moisture content can be increased with a 48-hour soak from 35.2 to 36.8%, but it is not expected that this difference will affect dormancy breakage, germination capacity or rate. The average GC was greater with the 24-hour soak (80 *vs.* 78%), but not significantly different from the 48-hour soak. The greatest source of variation was between seedlots that showed statistically significant differences in moisture content (before and after stratification) and in GC. The results are based on a sample of ten seedlots distributed throughout the BC range of the species (Table 1).

With the operational-sized samples the 48-hour soak without surface drying was the best treatment. Analysis of variance indicated that the 48-hour soak was superior (significantly different), but that the effect of surface drying was not statistically significant. The average advantage of the 48-hour soak was an increase in GC of 2.2% for four seedlots of Py.

These results possibly reflect one of the greatest fears in seed technology – the disagreement between lab testing and operational testing in the statistical significance of a term in the model (i.e. Soak Duration). In this case lab results indicate no advantage to increasing soak duration to 48 hours, while operational results indicate an advantage. The experimental design was not identical between the two areas, but they both did consider soak duration in Py.

I am recommending that we retain the current 24-hour soak for Ponderosa pine at this point in time. The lab studies were based on a larger sample of seedlots (which could not be practically replicated with operational quantities of seed), large falldowns occurred with a 48-hour soak in lab testing and the fact that the operational germination gain was rather small at 2.2% have influenced this decision. Surface drying is not performed in lab testing, but it was thought that this practice in seed preparation might have been excessive causing the relatively low stratification moisture contents found in Quality Assurance Py monitoring. Rather than change the soak duration – more

[3] the interaction terms include – Seedlot* Soak; Seedlot* Surface Dry; Soak*Surface Dry and Seedlot*Soak*Surface Dry. The full Analysis of Variance models and statistical tables have not been included for brevity, but will be made available by the author on request.

emphasis will be placed on limiting the amount of surface drying occurring on the request prior to stratification. I believe that the differences exhibited between the lab and seed preparation are operationally quite small, although they are statistically significant. A larger more consistent difference would need to appear before a change in methods is justified.

Nurseries stratifying there own seed may want to extend soak to 48 hours and clients can request this service of the Tree Seed Centre at the beginning of the sowing season. Ponderosa pine can also have relatively high levels of *Fusarium* sp. contamination [56% of seedlots infected with an average infection rate of 2.1%] and a running water soak or other seed sanitation treatment should be incorporated to limit losses from this pathogen. The results clearly indicate the significance of seedlot differences – we should all become more familiar with individual seedlots. Check the fungal assay results included on the sowing request label and available on SPAR[4]. If anyone would like detailed results of seedlots used in this trial, please contact me.

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[4] **SPAR** = **S**eed **P**lanning **A**nd **R**egistry system.