REFEREED RESEARCH ARTICLE

Refinement and Stratification of Thinleaf Alder and Water Birch Seeds from New Mexico

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ABSTRACT

For multiple seed collections of thinleaf alder (*Alnus tenuifolia* (Nutt.) Breitung [Betulacae]) and water birch (*Betula occidentalis* (Hook.) [Betulacae]), response to IDS (Incubation, Drying, and Separation), gravity separation, and stratification was highly variable among seed collections. In thinleaf alder, drying periods of 18 or 24 h following a 24-h incubation period were comparable to dry seed separation in petroleum ether for increasing percentage of filled seeds. In water birch, IDS treatments resulted in lower percentages of filled seeds than separation in 95% ethanol. Overall, cold (5 °C [41 °F]) wet stratification for 56 d improved water birch germination from 11% to 16%. In thinleaf alder, response to a 56-d stratification ranged from 0% to 16% germination improvement. Using separated seed in combination with appropriate stratification length achieved the largest improvements in germination. Treatment selection is discussed in relation to optimizing use of limited greenhouse space and seed supply.

KEY WORDS: IDS separation, gravity separation, stratification

NOMENCLATURE: ITIS (2001)

Thinleaf alder (*Alnus tenuifolia* (Nutt.) Breitung [Betulacae]) occurs in mountain ranges of western North America, typically growing as a shrub or small tree in riparian areas at elevations of 1520 to 3040 m (5000 to 10,000 ft) (Vines 1960). Thinleaf alder fixes atmospheric nitrogen via a symbiotic relationship with the actinomycete *Frankia* spp. (Virtanen 1957; Bond 1976) and the species clump-forming habit is valuable in erosion control and disturbed land revegetation (Vines 1960).

Water birch (*Betula occidentalis* (Hook.) [Betulacae]) occurs naturally from southern California and New Mexico north to Alaska, Manitoba, and North Dakota, but is absent along the Pacific Coast mountain ranges and portions of the Sierra Nevada Mountains (Uchytil 1989). It occurs as a shrub or small tree along streams or in moist canyons, and occasionally on dryer sites of the mountain West at elevations of 1500 to 2700 m (4900 to 8900 ft) (Vines 1960).

Interest in these species for revegetation applications has recently increased because both species grow fast, produce prolific amounts of seeds, and have short life cycles (Elias 1980). While propagation of alder and birch species has been studied, literature pertaining to propagation requirements of thinleaf alder and water birch is lacking.

Seeds of Betulaceae are characteristically very small and light (1500 to 2500 seeds/g [42500 to 70800

seeds/oz]) and may have a winged integument for wind dispersal (Vines 1960). Seed quality and germination capacity are often very low, as it is difficult to separate sound from empty seeds using standard size and density separation techniques on small, winged seeds (Brinkman 1974; Schopmeyer 1974).

The IDS method (Incubation, Drying, and Separation) for separating viable filled seeds from unfilled or nonviable seeds has been successful for both coniferous and hardwood species (Simak 1983; Sweeney and others 1991; Downie and Wang 1992; Falleri and Pacella 1997). In the IDS method, after imbibition, empty or nonviable filled seeds lose water more rapidly than viable filled seeds during drying. The differential moisture content during drying allows separation by flotation or other density separation methods.

Alnus and *Betula* seeds exhibit various degrees of dormancy that can be broken by cold stratification and/or germination under red light (Brinkman 1974; Schopmeyer 1974; Dirr and Heuser 1987; Young and Young 1992). Pretreatment requirements for germination of alder seeds are variable between and within species. Stratification periods of 60 to 180 d are recommended for many alder species (Dirr and Heuser 1987). However, stratification treatment of thinleaf alder did not improve germination percentage (Young and Young 1992). The purpose of our study was to determine the effectiveness of IDS and gravity separation techniques to increase the percentage of filled seeds in thinleaf alder and water birch. Secondly, we examined using separation techniques in combination with varying levels of stratification on germination of thinleaf alder and water birch. To achieve these objectives, 2 experiments using multiple seed collections of both thinleaf alder and water birch from the southern Rocky Mountains were conducted. First, a refinement experiment tested the effects of multiple separation treatments on percentage of filled seeds generated in sinking and floating fractions and the ability of these treatments to recover filled seeds in the original samples. Secondly, a germination experiment tested the efficacy of separation and stratification treatments on germination.

MATERIALS AND METHODS

Seed Collections

Thinleaf alder strobiles were collected in October and November of 1998 in Catron County, New Mexico, near Luna (Cottonwood Canyon Campground) and Reserve (Head of the Ditch Campground) and in Taos County, New Mexico (Red River Canyon near the Molycorp molybdenum mine) (Table 1). Seed lots from Red River Canyon (RRC, Moly) included collections from distinct stands within a 4-km (2.5-mile) stretch of the canyon. Bracts were collected when <10% of the strobiles were beginning to open. The Luna, Reserve, and both Red River seed collections

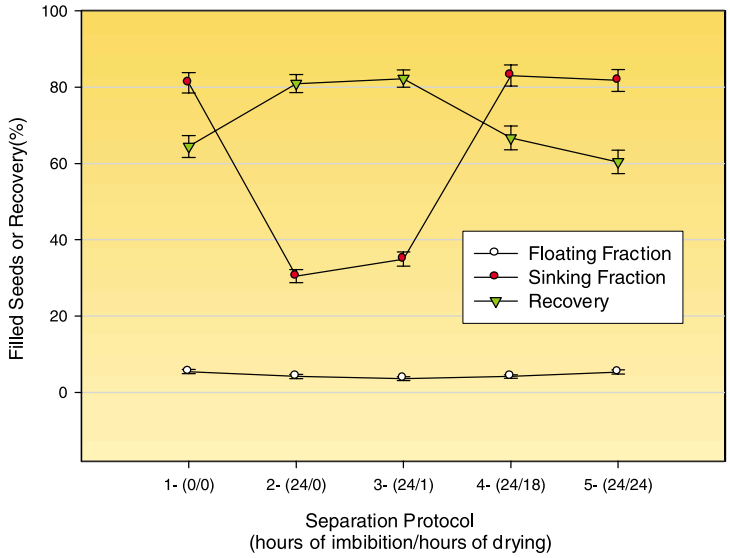

Figure 1 • *Effect of separation protocol on percentage of filled seeds in sinking and floating fractions, and percentage recovery of filled seeds present in the original sample by the sinking fraction for thinleaf alder. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.*

(RRC-1, RRC-2) of thinleaf alder were used in the refinement study. The Luna, Reserve, and RRC (RRC-1 and RRC-2 pooled) collections and a commercial seed collection, collected in fall 1998 in Chaffee County, Colorado, were used in the germination study. Strobiles were kept cool and allowed to dry for several weeks. Thinleaf alder seeds were separated from opened strobiles by rubbing on a coarse screen.

Birch strobiles were collected in October and November of 1998 in Taos County, at 4 locations (RRC-3, Moly-1, Moly-2, and Moly-3) in the Red River Canyon near the Molycorp molybdenum mine (Table 1). Bracts were collected when < 10% of the strobiles were beginning to open. All 4 collections were used in the refinement study. The RRC (RRC-3 and Moly-3 pooled), Moly-1, and Moly-2 collections of water birch along with commercial seeds collected in fall 1998 in Chaffee County, Colorado were used in the germination study. Water birch strobiles were kept cool and allowed to dry for several weeks, allowing seeds to release from bracts.

Prior to any treatments, all seed collections were examined for percentage of filled seeds (baseline percentage of filled seeds) using a dissecting microscope at 30X magnification (Berry and Torrey 1985). Baseline percentage of filled seeds for thinleaf alder was estimated by averaging the results of 25 samples of 100 seeds for each seed collection (Table 1). Baseline percentage of filled seeds for water birch was determined by averaging the results of 15 samples of 50 seeds for each seed collection (Table 1). For both species, working samples were drawn from seed collections by mixing the entire collection thoroughly, and halving and re-

TABLE 1

Seed collection locations and baseline percentage of filled seed for thinleaf alder and water birch seed used in experiments

Species	Collection name	Baseline percentage of filled seed	Location description	Elevation (meters) [a]	Latitude longitude [b]
Thinleaf Alder	Luna	23	Head of the Ditch Campground	2134	N33°49′ W108°59′
	Reserve	27	Cottonwood Canyon	1829	N33°37′ W108°55′
	RRC-1	1	Red River Canyon (river bottom)	2377	N36°41.08′ W105°31.52′
	RRC-2	1	Red River Canyon (up slope)	2492	N36°41.67′ W105°29.85′
	Chaffee	54	West of Poncha Springs, Colorado	na	N38°31′ W106°05′
Water Birch	RRC-3	7	Red River Canyon	2377	N36°41.081′ W105°31.52′
	Moly-1	4	Molycorp-tailings road	2361	N36°41.23′ W105°32.36′
	Moly-2	5	Molycorp-lower overburden pile	2492	N36°41.67′ W105°29.85′
	Moly-3	6	Molycorp-Safety Berm	2403	N36°40.86′ W105°32.36′
	Chaffee	30	West of Poncha Springs, Colorado	na	N38°31′ W106°05′

[a] Conversion: 1m = 3.3 ft.

[b] Latitude and longitude values for the Luna, Reserve, and Chaffee collections were determined from a topographical map and recorded to the nearest whole minute. Values for the RRC and Moly collections were obtained with a global positioning receiver and recorded to the nearest 100th of a minute.

halving the collection until the necessary quantity of seeds was obtained. Fifty or 100-seed samples were counted out of the working sample following a thorough remixing, and these samples were randomly assigned to treatments.

Separation Media

Initial trials with ethanol (specific gravity = 0.79) and water were unsuccessful at separating filled and unfilled thinleaf alder seeds, either using IDS or when separating dry seeds. In both cases, filled and unfilled seeds remained in the floating fraction. Initial trials showed that petroleum ether (specific gravity = 0.60) was more effective as a separation medium.

Gravity separation in water was ineffective for separating water birch seeds. However, both ethanol and petroleum ether effectively separated dry water birch seeds. Petroleum ether, ethanol, and water were somewhat effective in separating water birch seeds previously treated by the IDS method. Ethanol was chosen as the separation medium because of cost, effectiveness, and availability.

Seed Refinement Study

Separation treatments for thinleaf alder seeds included density separation of dry seeds in petroleum ether (control) and IDS separation of 24-h imbibed seeds in petroleum ether following drying periods of 0, 1, 18, or 24 h. Five replications of 100 seeds were performed for each treatment.

Separation treatments for water birch seeds were density separation of dry seeds in 95% ethanol (control), and IDS separation of 12-h imbibed seeds in 95% ethanol following drying periods of 0, 0.5, 1, and 2 h. Three replications of 50 seeds were performed for each treatment.

All seeds were imbibed by submersion in a 38-l (10-gal) glass aquarium filled with distilled water and equipped with an aeration pump and filter. Seeds were packaged in filter paper and enclosed in weighted wire cages to keep them submerged. Following imbibition, seeds were thoroughly blotted and placed on clean filter paper. The drying incubation was performed in a closed chamber consisting of a 38-l (10-gal) aquarium with polyethylene film taped over the top. Constant humidity inside the chamber was obtained using $CaCl_2 \cdot 6H_2O$ salt in a saturated solution prepared by adding 5000 g $CaCl_2 \cdot 6H_2O$ to 3.0 l of distilled water (Young 1967; Slavik 1974). Seeds were placed on filter paper and suspended on a screen above the solution. Humidity remained at 50% and was monitored using a hygrometer.

After IDS drying, seeds were placed briefly in petroleum ether or ethanol and the solution was vigor-

ously stirred for 20 s to separate seeds. Floating seeds were removed from the surface, rinsed, and placed on clean, moistened filter paper within plastic bags. Sinking seeds were strained through a net and packaged in a similar manner. Percentage of filled seeds in each fraction was determined by dissection. Percentage of the total filled seeds in the original sample recovered in the sinking fraction (percent recovery) was calculated using Equation 1.

Equation 1:

$$\text{Percent Recovery} = \frac{\text{Number of filled seeds in sinking fraction}}{\text{Number of filled seeds in sinking fraction} + \text{Number of filled seeds in floating fraction}} \times 100$$

Germination Study

This study tested the factorial combination of separation, collection, and stratification treatments for both species. The 3 seed separation treatments used for thinleaf alder were: 1) the floating seed fraction following 24-h imbibition and 18-h drying using petroleum ether; 2) the sinking fraction using the seperation above; and, 3) seed imbibed for 24-h with no separation. Seed separation treatments used for water birch included: 1) the floating seed fraction following 12-h imbibition using 95% ethanol; 2) the sinking fraction following 12-h imbibition using 95% ethanol; and, 3) seed imbibed for 12-h with no separation.

For thinleaf alder, we used stratification lengths of 0, 28, and 56 d, while for water birch stratification lengths were 0, 21, and 56 d. For stratification, seeds were placed between layers of paper towel, moistened with 25 ml (0.8 fl oz) of distilled water, and sealed in polyethylene bags. Bags were stored in a cooler with an average temperature of 5 °C (41 °F) (temperature ranged from 4.0 to 6.0 °C [38 to 43 °F]) for the respective treatment durations. Initiation of stratification treatments was staggered so that all treatments came out of stratification concurrently.

The factorial arrangement of collection, seed separation, and stratification treatments resulted in 36 treatment combinations for each species. Each treatment combination was replicated with four, 100-seed samples. Following stratification, seeds were sown in Ray Leach Super Cells (Steuwe & Sons Inc; Corvallis, Oregon) containing a 2:1:1 ratio by volume of peat:perlite:vermiculite with Osmocote 14N:14P_2O_2:14K_2O slow release fertilizer at a rate of 4 kg/m^3 (6.75 lb/yd^3). Five seeds were sown per container with 80 containers per treatment combination. Containers were placed in the center of a 10 m (33 ft) by 13 m (43 ft) greenhouse for germination. Treatments were arranged in a randomized complete block design with 4 blocks per species. Each block included one, 20-container (100-seed) replication of each treatment combination. Germination conditions were ambient light (average 13.5 h/d and 70% relative humidity, with an average daytime temperature of 24 °C (75 °F) (daytime temperature range 20 to 27 °C [68 to 80 °F]), and an average night temperature of 22 °C (70 °F) (nighttime temperature range 20 to 23 °C [69 to75 °F]). Cells were watered at 2-h intervals 6 times daily. Germination was recorded 7, 14, 21, and 28 days after planting.

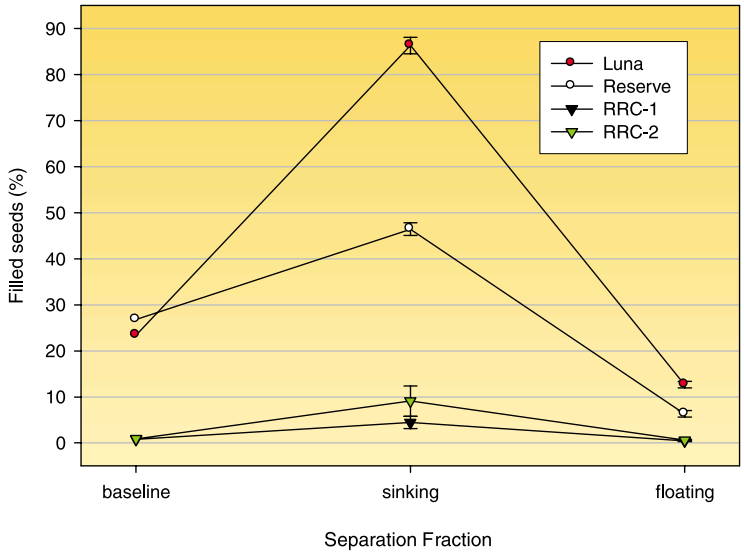

Figure 2 • *Effect of separation treatment on thinleaf alder percentage of filled seeds by seed collection. Baseline percentage of filled seeds is included for reference.*

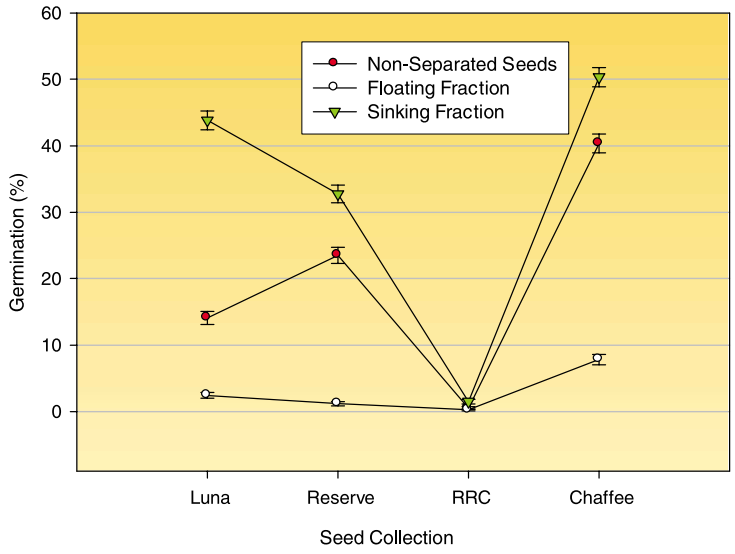

Figure 3 • *Effect of separation treatment on thinleaf alder germination by seed collection. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.*

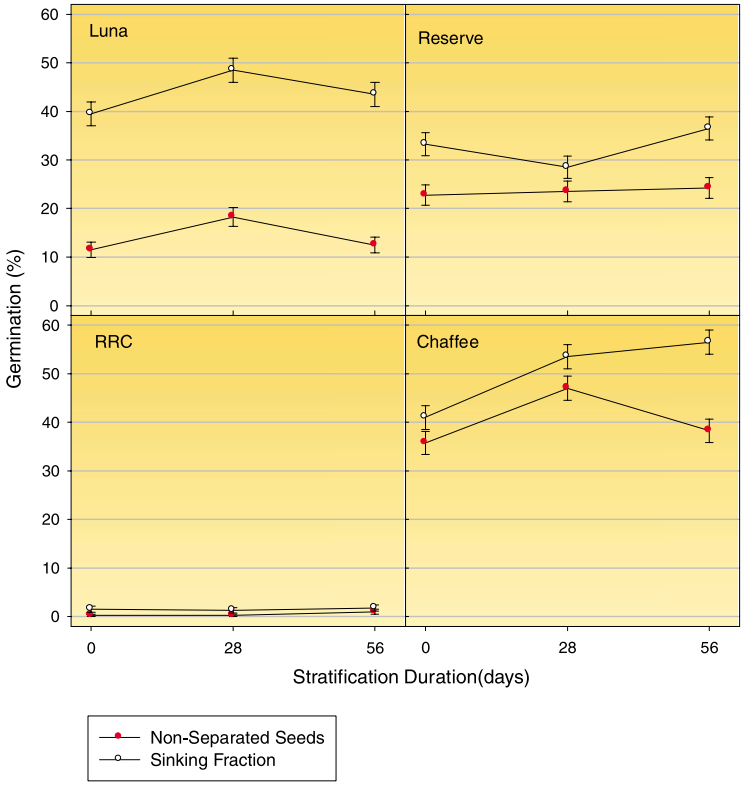

Figure 4 • *Thinleaf alder germination percentage as influenced by stratification length, separation treatment, and seed collection. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.*

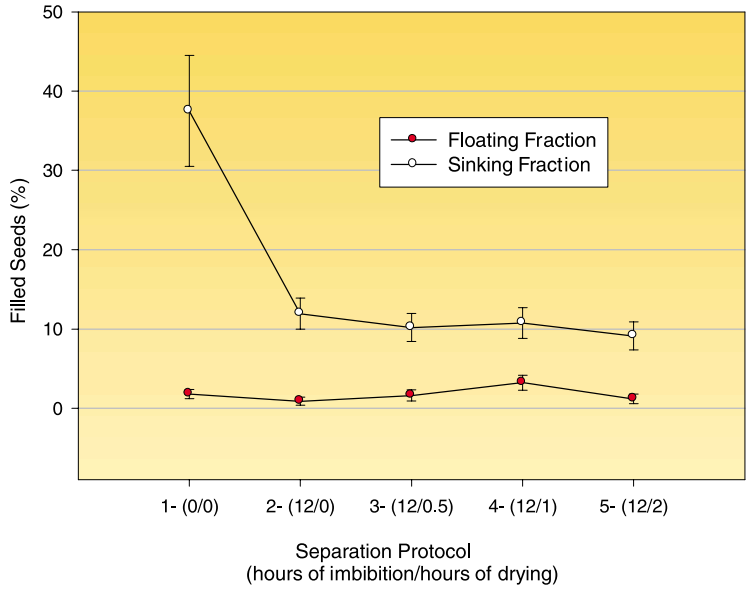

Figure 5 • *Water birch percentage of filled seeds as influenced by separation protocol and separation fraction. Error bars represent ± one standard error. Error bars are too small to be visible in some cases. Protocols are described in Table 2.*

Data Analysis

Categorical analysis of variance using SAS PROC CATMOD (SAS Institute 1989) was performed on all data. Categorical analysis of variance fits linear models to functions of response frequencies, and partitions the variation among those functions into various sources (SAS Institute 1989). This procedure is appropriate for binomial type of probability distribution (seeds filled or not filled) and does not require data transformation.

For the refinement study, the response variables were percentage of filled seeds in the sinking and floating fractions, and the percentage of filled seeds recovered from those present in the baseline sample. Data were analyzed as a 5 (preparation protocol) by 2 (separation fraction) by 4 (seed collection) factorial for each species. For the germination study, the response variable was germination percentage, and data were analyzed as a 3 (separation treatment) by 3 (stratification duration) by 4 (seed collection) factorial for each species.

For both experiments, marginal percentages (main effect and interaction combinations) along with standard errors were calculated using PROC MEANS (SAS Institute 1989). Pairwise Z-tests ($\alpha = 0.05$) were used to separate mean percentages. This method of percentage separation is analogous to Fisher's LSD.

RESULTS

Thinleaf Alder

Refinement Study

Preparation protocol, seed collection, separation fraction, and interactions between fraction and collection and fraction and protocol influenced the percentage of filled seeds. The percentage of filled seeds was low in the floating fraction (mean = 4%) and varied in the sinking fraction across preparation protocols from 30% to 83% (Figure 1). The control and the two IDS treatments with the longest drying durations (18 and 24 h) had higher percentages of filled seeds in the sinking fraction than IDS treatments without drying or a 1-h drying period. Overall, floating fractions had a lower percentage of filled seeds (4%) than sinking fractions (47%).

Seed collections differed in baseline percentage of filled seeds, percentage of filled seeds in the sinking fraction, and percentage of filled seeds in the floating fraction (Figure 2). Separation improved the percentage of filled seeds in the sinking fraction compared to the baseline by almost 4X for the Luna collection, 2X for the Reserve collection, 6X for the RRC-1 collection, and 10X for the RRC-2 collection. In contrast to the percentage of filled seeds in the sinking fraction, percent recovery was increased with IDS treatments having either no drying or 1-h drying (Figure 1). Percent recovery also varied among collections, 32% for RRC-2, 52% for RRC-1, 54% for Luna, and 88% for Reserve.

Germination Study

Seed collection, separation treatment, the interaction of both these factors, and the 3-way interaction of stratification length, separation treatment, and seed collection affected germination percentage. Averaged over all treatments, the RRC seed collection germinated poorly (1%) compared to other seed collections, while the Chaffee seed collection had the highest germination (33%). The Reserve and Luna seed collections germinated at 19% and 20%, respectively. This result follows baseline percentages of filled seeds for these collections (Table 1). Seeds in the sinking fraction had the highest germination at 32%, compared to 3% for the floating fraction and 19% for non-separated seed. The effect of separation treatments varied among seed collections (Figure 3). Seed collections with higher overall germination percentages (and baseline filled percentages)—Luna, Reserve, and Chaffee—also had the largest improvements in germination, 30%, 9%, and 10%, respectively. Germination of the RRC-1 seed collection was poor regardless of separation treatment, however, this collection did have a significant 1% improvement in germination in the sinking fraction. Some germination occurred in the floating fraction, with the Chaffee collection having the greatest germination in this fraction (8%).

The influence of stratification on germination was both seed collection- and separation fraction-dependent. In the Chaffee collection the germination response to stratification differed for the sinking and non-separated fractions. For seeds in the sinking fraction, both 28- and 56-d stratification periods improved germination, whereas only 28-d of stratification improved germination in non-separated seeds (Figure 4). This later response is similar to the trend observed in both non-separated and sinking fractions of seeds in the Luna collection. Stratification did not significantly affect germination in either fraction of the Reserve or RRC collections of thinleaf alder.

Water Birch

Refinement Study

Preparation protocol, separation fraction, and the 2-factor interactions between separation fraction and seed collection and separation fraction and protocol influenced the percentage of filled seeds. As was the case with thinleaf alder, percentage of filled water birch seeds was low in the floating fraction, but varied with the preparation protocol in the sinking fraction (Figure 5). All 4 IDS treatments reduced the percentage of filled seeds in the sinking fraction to less than one-third that of the non-imbibed control. Seed collection also influenced percentage of filled seeds in the 2 fractions. Moly-2 and Moly-3 seed collections had higher percentages of filled seeds in the sinking fraction than the RRC-3 and Moly-1 collections (Figure 6). Overall, the sinking fraction had a higher percentage of filled seeds

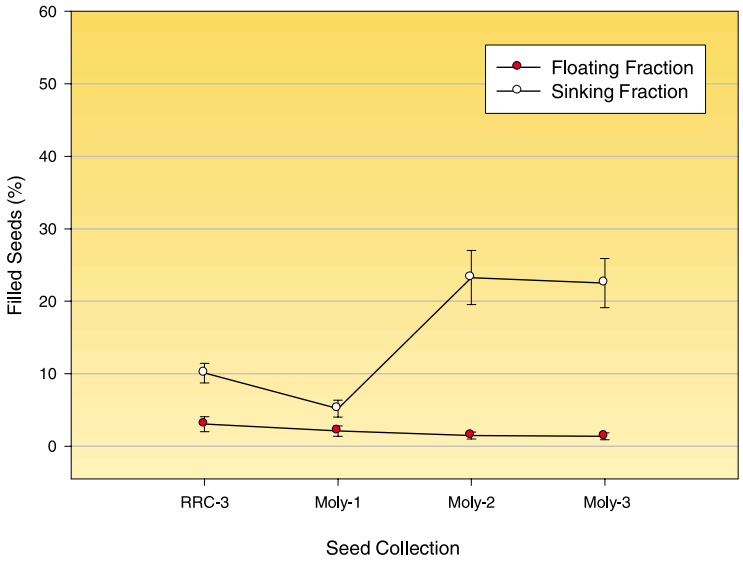

Figure 6 • *Water birch percentage fill as influenced by seed collection and separation fraction. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.*

(12%) than the floating fraction (2%). Neither preparation protocol nor seed collection affected the percent recovery. Percent recovery for the various protocols ranged from 64% to 91%. Mean percent recovery for the 4 seed collections ranged from 70% to 89%.

Germination Study

Stratification, separation treatment, seed collection, and all interactions of these factors affected water birch germination. On average, seeds in the sinking fraction had the highest total germination (33%) compared to the floating fraction (1%) and non-separated seeds (7%). Separation improved germination by 2X up to over 10X depending on seed collection (Figure 7). Mean germination varied from 5% for Moly-1 to 13% for RRC-1, 15% for Moly-2, and 19% for Chaffee.

Increased stratification length improved germination slightly, from 11% for non-stratified seeds up to 16% for seeds stratified 56 d, but only seeds in the sinking fraction responded positively to the 56-d stratification treatment (10% increase in germination). The interaction between stratification and separation treatments was inconsistent across seed collections (Figure 8). Germination of non-separated seeds was unaffected by stratification. Sinking fractions of different collections, however, varied considerably in response to stratification. In the Moly 1 collection, the 21-day stratification had no impact, while 56 d of stratification improved germination by 7X. However, in the Moly-2 and RRC collections, germination peaked at 21 d of stratification. Both 21 and 56 d of stratification reduced germination in seeds from the sinking fraction of the Chaffee collection. Seeds in floating fractions were unaffected by stratification treatments.

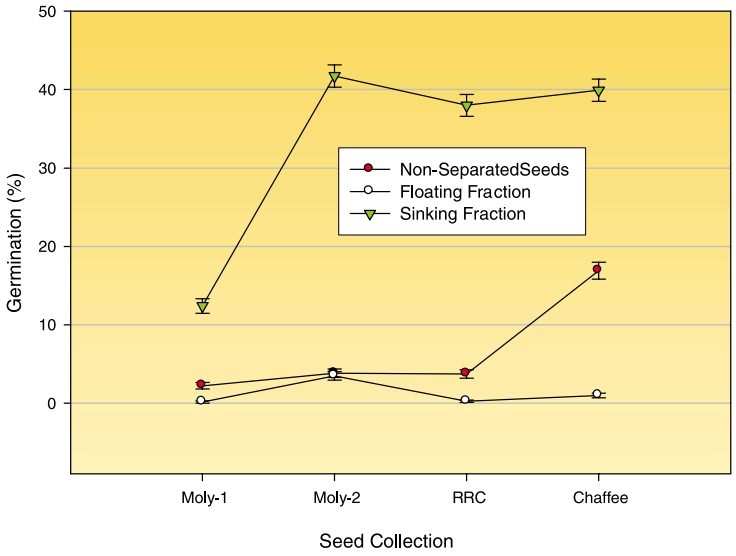

Figure 7 • *Effect of separation treatment on water birch germination by seed collection. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.*

DISCUSSION

Seed refinement techniques must not only increase the percentage of filled (potentially viable) seeds within seed collections but also must increase the percentage of viable seeds as measured by viability testing or, as was the case in this study, germination. In addition, the benefit of an increased percentage of viable seeds in the sinking fraction must not be overweighed by the loss of viable seeds in the floating fraction. Although the 50% relative humidity used in this study is higher than is standard for the procedure, this level was chosen to ensure procedural repeatability at relative humidities higher than in the Chihuahuan desert of southern New Mexico where this study was conducted. Downie and Wang (1992) found that for 3 coniferous species, drying seeds at 50% relative humidity achieved as great a difference in moisture content between live and dead seeds as did drying at 20% relative humidity. In our study, the efficacy of multiple IDS and gravity separation treatments was first assessed by the percentage of filled seeds. The most promising separation treatments were then evaluated across a range of stratification lengths to ensure that improved fill corresponded to improved germination of both species.

Gravity separation of non-imbibed seeds for both thinleaf alder and water birch was superior to IDS treatments in improving the percentage of filled seeds in the sinking fraction. The 2 alder IDS treatments with the longest drying times, 18 and 24 h, resulted in percentages of filled seeds in sinking fractions similar to those following gravity separation of non-imbibed seeds. It is possible that an intermediate duration of drying (between 1 and 18 h) would have improved the percentage of filled seeds in the sinking fraction. The shorter drying times (0 and 1 h) may have been insufficient to allow unfilled seeds to lose moisture, while the 18- and 24-h drying times may have allowed filled seeds to lose most of the imbibed water. The IDS protocol used in the second experiment improved thinleaf alder germination, with greater improvement in the better quality seed collections. This response is consistent with the results of the refinement study in which the percentage of filled seeds was increased when separation techniques were employed.

For water birch, IDS drying times of 0.5 to 2 h may have been too short to allow unfilled seeds to lose sufficient moisture. Insufficient drying results in an increase in the percentage of unfilled seeds in the sinking fraction. The assumption was made that water birch seeds, being small with large integuments, would lose imbibed water at a fast rate. This may not have been the case, as indicated by the higher percentage of empty seeds in the sinking fraction.

The influence of drying time on the efficacy of IDS treatment has been seen in other species. For London plane tree (*Platanus* x *acerifolia* (Alton) Willd.), drying times from 7.5 h to 24 h improved germination percentage of the sinking fraction (and filled seed percentage) beyond that of the control, but only seeds receiving 24 h of drying as part of an IDS treatment had greater germination than non-treated seeds separated in petroleum ether (Falleri and Pacella 1997).

In our study we observed considerable variability among seed collections in response to separation treatments. This difference was most pronounced between the Reserve and Luna seed collections of thinleaf alder—seed collections with similar baseline percentages of filled seeds. Separation increased the percentage of filled seeds for the Luna collection to a greater extent than for the Reserve collection. Differences in the rate of moisture loss between the Luna and Reserve collections may have existed during the drying portion of the IDS regimes. Differences among seed collections in response to similar seed refinement techniques (IDS) have been observed in other studies (Donald 1985; Downie and Wang 1992).

During seed refinement, some viable filled seeds are lost in the floating fraction (Sweeney and others 1991; Downie and Wang 1992; Falleri and Pacella 1997). The percentage of filled seeds from the original sample recovered in the sinking fraction provides a measure of how efficient the refinement technique is at reducing the number of filled (potentially viable) seeds lost in the floating fraction. For thinleaf alder, high recovery of filled seeds was inversely related to the IDS treatment's ability to remove unfilled seeds. Taylor and Kenny (1985) found a similar trend in an attempt to upgrade germinated cabbage (*Brassica oleracea* L.) seeds using density gradients. As percent recovery increased, germination percentage in the sinking fraction decreased because of the increased recovery of nongerminable

seeds. In the case of water birch, separation technique did not impact percent recovery but did impact the percentage of filled seeds in the sinking fraction.

Effects of stratification on germination of both thinleaf alder and water birch were most pronounced on the sinking fractions of separated seeds, as would be expected, because those fractions contain the highest percentages of filled, viable seeds. In this fraction, the effect of stratification varied among collections of both species. Stratification appears to be advantageous for many species of alder, but the response to stratification can be highly source specific (Schrader and Graves 2000). In paper birch (*Betula papyrifera* Marsh.), New Hampshire and Alaska seed sources have been shown to have different optimum durations of stratification (Bevington and Hoyle 1981). Provenance variation in seed properties and germination is not uncommon and has been reported for a wide range of woody species (Young and Young 1992; Baskin and Baskin 1998).

Depending on a grower's constraints, either greenhouse space or seed supply, the evaluation of seed refinement techniques could be based on 1 of 3 criteria: percentage of filled seeds in the sinking fraction, percentage of filled seeds recovered, or the product generated by multiplying these 2 values. In cases where seed supply is a greater constraint, selection of seed refinement technique may be based solely on the percentage of filled seeds recovered. This seed refinement technique may be less efficient in removing unfilled seeds, but loss of filled seeds would be minimized. In the case where growing space is the greater constraint, the percentage of filled seeds in the sinking fraction would determine the selection of seed refinement technique. When both greenhouse space and seed supply are limited, the product of multiplying percentage of filled seeds in the sinking fraction by the percentage of filled seeds recovered may be used to determine the appropriate protocol to use. The use of this information in conjunction with spreadsheet-based seed sowing programs allows nursery managers to select the best seed refinement technique for their nursery (Wenny 1993; Harrington and Glass 1997).

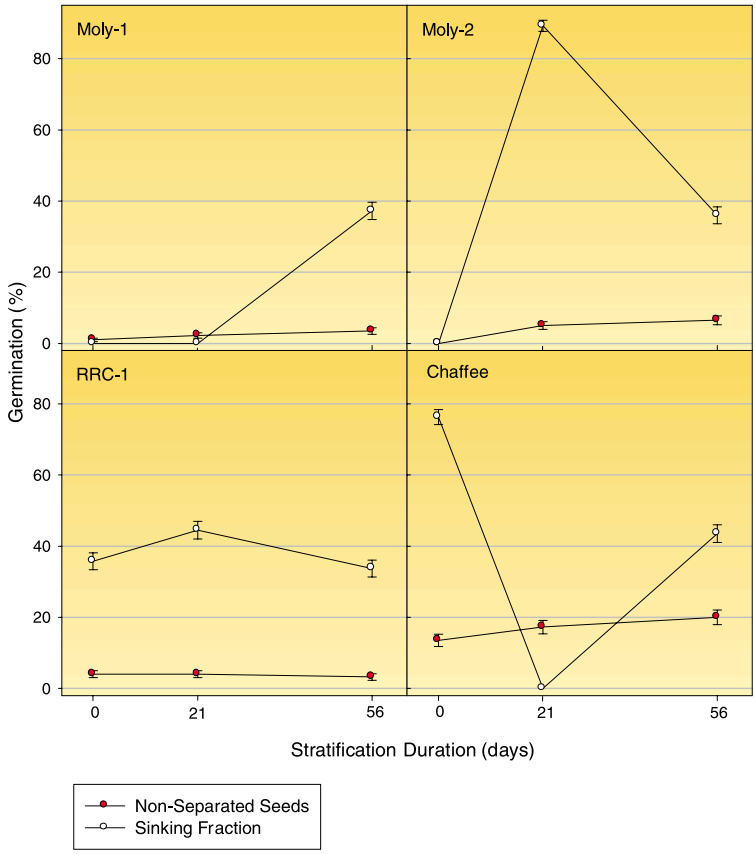

Figure 8 • *Water birch germination percentage of non-separated and sinking fractions as influenced by stratification length, seed collection, and separation fraction. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.*

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