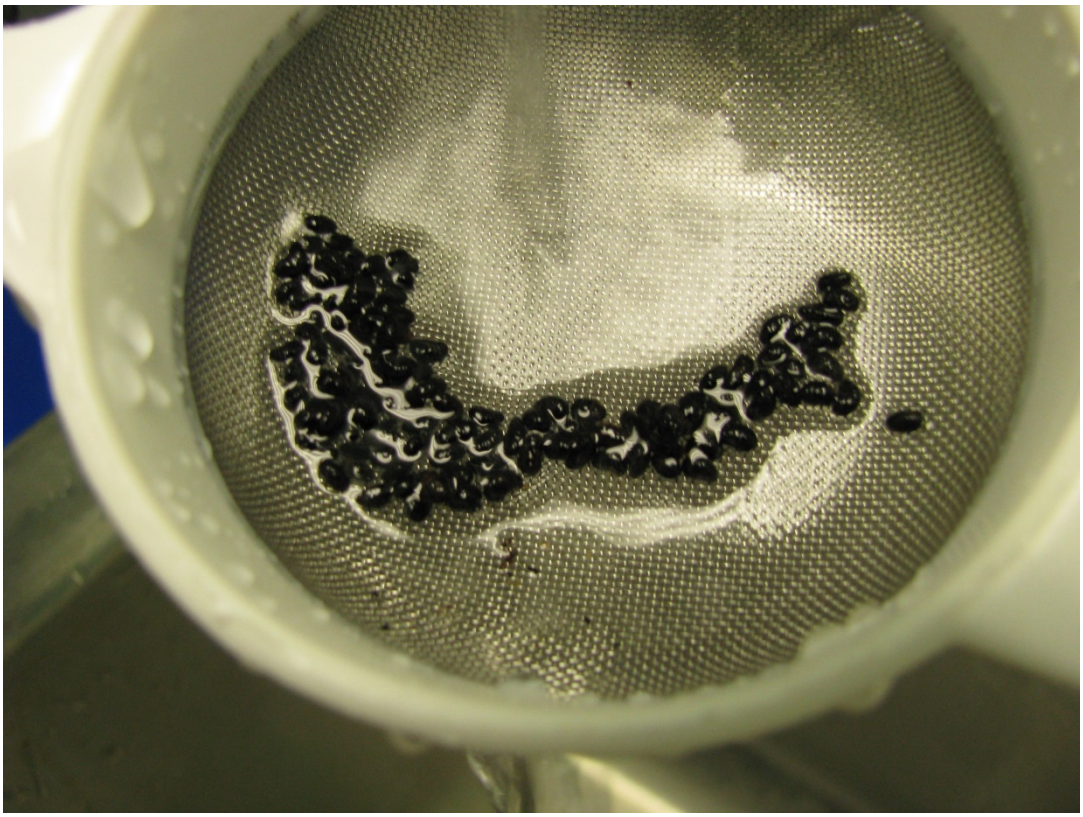
Development of propagation protocols for Bartonberry (*Rubus bartonianus*)



2014

Progress Report to the USDI Bureau of Land Management, Vale District

Report prepared by Matt A. Bahm and Erin C. Gray

Institute for Applied Ecology

PREFACE

This report is the result of an agreement between the Institute for Applied Ecology (IAE) and a federal agency. IAE is a non-profit organization whose mission is conservation of native ecosystems through restoration, research and education. Our aim is to provide a service to public and private agencies and individuals by developing and communicating information on ecosystems, species, and effective management strategies and by conducting research, monitoring, and experiments. IAE offers educational opportunities through 3-4 month internships. Our current activities are concentrated on rare and endangered plants and invasive species.

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Cover photograph: *Rubus bartonianus* seeds following sulfuric acid treatment, prior to washing to remove residue and placement in stratification.

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TABLE OF CONTENTS

Development of propagation protocols for Bartonberry (*Rubus bartonianus*)

PROGRESS REPORT TO THE USDI BUREAU OF LAND MANAGEMENT, VALE DISTRICT

INTRODUCTION

Bartonberry (*Rubus bartonianus*) is a narrow endemic that occurs in Oregon and Idaho in the mid sections of Hells Canyon of the Snake River and its tributaries. Historically, Bartonberry occurred over 59.5 river miles in Hells Canyon. Recent field surveys in 2009 and 2010 were unable to relocate the southern and northern most Bartonberry locations, thus, shrinking the global distribution of Bartonberry by 14.5 river miles (24%). The ultimate goal of this project is to determine the requirements to grow out Bartonberry from seed and cuttings so that it may be reintroduced to the historic southernmost location for the species, to maintain its historic global distribution.

Figure 1. BLM Botanist, Roger Ferriel, collecting cuttings of *Rubus bartonianus* for propagation research

Bartonberry is a federal species of concern, and a candidate for listing by the state of Oregon (ORBIC 2013). Bartonberry was named by Morton Peck in 1934, honoring Lenora Barton, a rancher who found Bartonberry at Battle Creek in 1931 (USDI BLM 2010). Bartonberry (*Rubus bartonianus*) is classified in the subgenus *Anoplobatus* along with more widespread species including *Rubus parviflorus* (thimbleberry) and *Rubus deliciosus* (delicious raspberry), which occurs primarily in Wyoming, Colorado, New Mexico, and Oklahoma (USDA NRCS 2015). Bartonberry grows in ravines and talus/rocky slopes of Hells Canyon, in the Wallowa Whitman National Forest in Oregon and the Payette National Forest in Idaho.

Bartonberry is a non-prickly shrub with white showy flowers that produce a deep, red raspberry in May to June (Brooks et al. 1991, Figure 2).

Current threats to the species include climate change, competition from non-native species, livestock grazing, weed control particularly along roadsides, and climate change (USDI BLM 2010). Competition from the non-native Himalayan blackberry (*Rubus armeniacus*) is a great concern. This non-native species can colonize similar habitats to Bartonberry and potentially out-compete this rare species (USDI BLM 2010).

While many species in the *Rubus* genera are economically important fruit producers, information on the germination requirements for wild species are rarely available (Wada and Reed 2011a.). Germination of *Rubus* seeds is inhibited by both physical and physiological dormancy. *Rubus* seeds contain a thick seed coat that poses a physical barrier and along with internal dormancy that is regulated by biochemical processes (Wada and Reed 2011b.). Previous germination trials have suggested that use of sulfuric acid, followed by a period of warm and cold stratification is optimal for many *Rubus* species, however they have shown variability in response to various treatments, dependent upon physical seed properties (Wada and Reed 2011a). Members of the subgenus *Anoplobatus* have a distinctive seed coat structure, with a hole in the seed coat on one end of the seed (hilar-end hole) which has been shown to potentially promote higher rates of germination of unscarified seed in closely related species (Wada and Reed 2008, Wada and Reed 2011 a.). Given the variability seen in the response of wild *Rubus* species to germination trials, there is great need to understand the germination requirements of rare species such as *Rubus bartonianus* (Bartonberry), to mitigate for further losses in this rare species. Further, if seed is not readily available, understanding the steps needed to propagate this species from stem cuttings are necessary for potential conservation measures.

Figure 2. Bartonberry (*Rubus bartonianus*) in flower

METHODS

Seed Propagation

Seeds collected from the Idaho portion of Hells Canyon in 2013 were used in germination trials to determine the most successful method to germinate Bartonberry from seed. It is unknown if Bartonberry seeds require (chemical or mechanical) scarification, warm and cold stratification or both for germination. We conducted germination trials with four scarification treatments (control, mechanical, bleach, sulfuric acid) and three cold stratification treatments (60, 90, and 120 days in cold) in combination (Figure 2). In total, we had 12 treatment combinations (Table 1). We started 550 seeds of each treatment (6,600 total seeds) which were each staggered by a month so that all seeds were removed at the same time (Table 2). For more detailed instructions, please see Appendix A.

Table 1. Treatment combinations for *Rubus bartonianus* germination protocol trials.

Seed treatments	
Scarification	**Warm stratification (20 °C)+ Cold stratification (1°C)**
No scarification- Control	30 days warm + 60 days cold
Mechanical	30 days warm + 90 days cold
Mild bleach solution	30 days warm + 120 days cold
Conc. Sulfuric acid	

Prior to mechanical scarification, seeds were tested with various levels of manual sanding to determine a good amount of scarification that would imbibe the seeds. From this we conducted mechanical scarification using a small mechanical scarifier, using the seeds from the trials as a reference. We scarified 110 seeds at a time using a small pneumatic seed scarifier, run for 3 minutes at 40 PSI. Following recommendations from USDA staff, the mechanical scarification treatment was followed by a treatment of 100% bleach for 15 minutes. This was to decrease pathogens that are present in the seed coat and could be released from mechanical treatment. Seeds were rinsed using a strainer and distilled water 3 times.

For the mild bleach treatment, all seeds were placed in a small beaker with 100% bleach covering the seed. Seeds were in the bleach for 60 minutes and were stirred occasionally. They were rinsed using a strainer and distilled water at least 3 times.

The sulfuric acid treatment was conducted at the USDA laboratory in Corvallis. Proper safety precautions and storage of chemicals are necessary given the strength of the sulfuric acid (98%). Seeds were placed in test tubes (110 to a tube) in an ice bath. A neutralizing solution was prepared using1.5 g of calcium hydroxide and 1.5 g of calcium hypochlorite dissolved into 500 ml of DI water, and placed aside. Each test tube was filled 1/3 full with sulfuric acid using a plastic pipette. Seeds were rinsed very quickly under flowing water. Because the sulfuric acid causes the seeds to become blackened and charred, seeds had to be rubbed to take the charred part off. They are then placed in a jar with the neutralizer solution, and left to sit for 30 minutes. After 30 minutes the jar was covered securely with cheesecloth and placed directly under flowing water so that the solution is quickly rinsed out and there is no more

white residue. Afterwards, the jars were placed in a bucket with water flowing into it for 30 more minutes. If seeds cannot be plated right away they can be stored for several days in DI water.

For each replicate, 550 seeds for each treatment were placed in sterilized small square germination trays on blotter paper on top of a piece of felt to hold moisture. Each tray held 100 seeds resulting in 5 trays with 100 seeds and 1 extra tray containing approximately 50 seeds. The number of seeds placed in each tray was noted on the label.

Table 2. Schedule of Bartonberry treatments in warm and cold stratification treatments

Replicate (4 treatments each)	Date into warm	Date into cold	Date removed
1	8/27/14 (30 days)	9/25/14 (120 days)	1/23/14
2	9/25/14 (30 days)	10/24/14 (90 days)	1/23/14
3	10/24/14 (30 days)	11/24/14 (60 days)	1/23/14

For each replicate, seeds trays were first placed in 30 days of warm stratification (20°C), as suggested by USDA scientists (Table 2). Seeds were checked on once each week and sprayed if necessary either with distilled water, or with 3% hydrogen peroxide solution if needed to abate fungal infection. After 30 days, trays were removed and placed into cold stratification (4°C) for either 120, 90, or 60 days. Many seeds germinated while in cold stratification. Trays were removed from cold stratification on 1/23/14 and were left in the greenhouse for one week to promote germination.

Potting of seeds occurred from 2/2/15-2/5/15. All trays were labelled with a unique ID, and we kept track of the original number of seeds in each tray, the number of live plants (those seeds that had already germinated), number of seeds potted, and number of dead (not potted). We potted seeds that looked like they were still viable and were not heavily infected by fungus. Seeds or germinants were potted into a 2:2:1 mix of coconut coir, perlite, and pumice, respectively, in SC10 superpots. We transferred each germination box into its own tray of pots, planting first the live plants, then the seeds. Some of the seedlings already had true leaves developing in the germination boxes at the time of potting. Trays filled with pots were marked with the number of days in cold stratification, treatment, and tray number.

Pots were watered daily and were fertilized weekly using a commercial 24,8,16 fertilizer. Pots were treated with predatory nematodes to combat fungus gnats and Deadline® for slugs, as necessary. Trays were moved every two weeks to ensure that trays were not differentially affected by certain parts of the greenhouse.

Figure 3. Bartonberry seeds in various treatments: (A.) Control, (B.) Mechanical, (C.) Bleach, and (D.) Sulfuric Acid

Cutting Propagation

Table 3. Treatment combinations for cutting propagation

State	Rooting Hormone	Media	Total cuttings
Oregon	Liquid	Coconut Coir	100
Oregon	Liquid	Pine Shavings	100
Oregon	Liquid	Perlite	100
Oregon	Liquid	Perlite:Vermiculite	100
Oregon	Powder	Coconut Coir	100
Oregon	Powder	Pine Shavings	100
Oregon	Powder	Perlite	100
Oregon	Powder	Perlite:Vermiculite	100
Idaho	Liquid	Coconut Coir	100
Idaho	Liquid	Pine Shavings	100
Idaho	Liquid	Perlite	100
Idaho	Liquid	Perlite:Vermiculite	100
Idaho	Powder	Coconut Coir	100
Idaho	Powder	Pine Shavings	100
Idaho	Powder	Perlite	100
Idaho	Powder	Perlite:Vermiculite	100
		Total	1600

Figure 4. Bartonberry cuttings in different rooting media: (left to right) coconut coir, sawdust, 1:1 perlite/vermiculite, perlite only

In November 2014 we travelled to Hells Canyon to collect cuttings for propagation to test the effectiveness of various rooting hormones and rooting media (Table 3). 200 large cuttings (1-2 ft in length) were collected from roadside sites in Idaho & 180 were collected from trailside sites in Oregon on 11/4/14. Cuttings were selected as a section of a stem that contained numerous nodes. Cuttings were kept moist and transported to the greenhouse at Oregon State University.

From the stems collected, we made 800 smaller cuttings each from Oregon and from Idaho, each containing at least 3 nodes. The bottom of each cutting was cut at an angle no more than 1in below a node. The angled end of each cutting was dipped in rooting hormone (either liquid or powder), and placed into a germination tray with 3 inches of media (perlite only, 1:1 perlite/vermiculite, pine shavings, and coconut coir, Figure 3). Media for the cuttings were based on recommendations from the literature (Rose et al. 1998). Coconut coir was used as a replacement for peat moss, which many commercial suppliers have discontinued carrying due to environmental concerns with sustainability. Cuttings were watered daily and media were sprayed with predatory nematodes to decrease issues with fungus gnats. Once calluses and or roots were formed, cuttings were transferred to SC10 superpots with a combination coconut coir, perlite, and pumice, at a ratio of 2:2:1, as suggested by staff at the USDA facility. Treatment combinations were kept separate in trays and were watered regularly.

RESULTS

Seed Propagation

We noted germination in all treatments during warm and cold stratification. We collected data on the treatment present for 120 days, and noted the earliest germination occurred in the mechanical scarification treatment under warm stratification (Table 4). This could be due to the abrasion of the seed coat that occurred with this treatment. Interestingly, we noted that 4 seeds germinated in the control treatment under warm stratification, suggesting that germination is possible, even without cold stratification or scarification.

Table 4. Number of plants that germinated during warm or cold stratification for seeds in cold stratification for 120 days.

Stratification	Bleach	Control	Sulfuric Acid	Mechanical
Warm	0	4	1	14
Cold	3	4	0	6
Total	3	8	1	20

After removal from cold stratification, germination occurred in all treatments, with variation becoming greater with increasing time in cold stratification (Figure 4). Most treatments (control, bleach, and sulfuric acid), had >64% germination after 60 days in cold stratification (Figure 4). The mechanical treatment had 35% germination after 60 days of cold stratification. This trend was consistent for 90 and 120 days of cold stratification for all treatments, except the mechanical treatment. In each of the two longer stratification treatments, mechanically treated seeds showed <43% germination. The sulfuric acid treatment consistently had the highest rates of germination, all >80%. Seeds in the bleach treatment also experienced high rates of germination (>73%). The control treatment, with less germination than the sulfuric acid and bleach treatments, was still relatively high with >60% of germination occurring (Figure 4). The numbers of dead seeds observed were similar across all treatments, except for the mechanical treatments that were in cold stratification for 90 and 120 days, where the number of dead was greater than all other treatments (Figure 4).

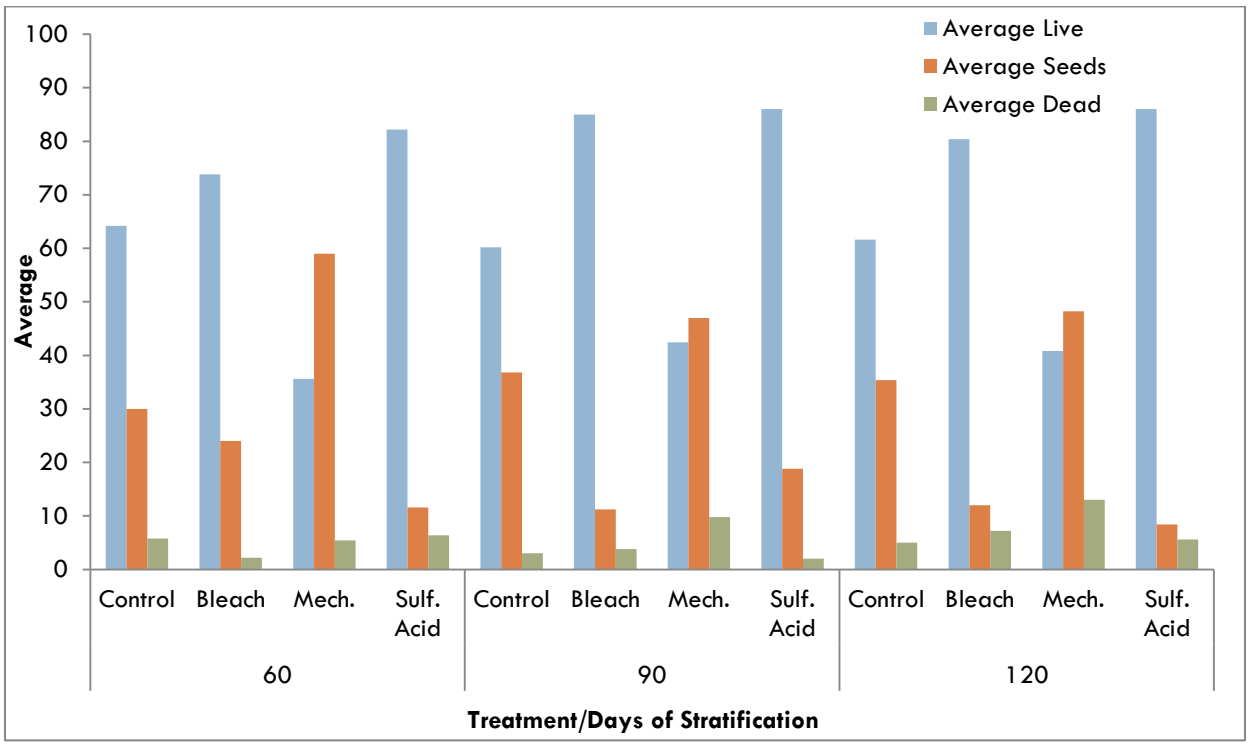

Figure 5. Average number (per 500 seeds) of live (germinated), seed (no germination), and dead seed for each of twelve treatment combinations for *Rubus bartonianus*.

Cutting Propagation

Formation of callus and/or roots varied by source population, media, and showed variation among rooting hormone in both the Oregon and Idaho populations (Figure 5). Perlite alone and in combination with vermiculite had greater callus/root formation than the other media tested during our study. The Idaho cuttings in the perlite mixes had >60% callus/root formation after 12 weeks. The Oregon cuttings also had higher callus/root formation in the perlite mixes, but showed variability between the liquid and dry rooting hormone (Figure 5). In the perlite media, the liquid rooting hormone resulted in 33% callus/root formation compared to 61% for the dry rooting hormone. A similar pattern was observed in the perlite vermiculite mixture, with 47% and 57% callus/root formation in the liquid and dry hormone, respectively.

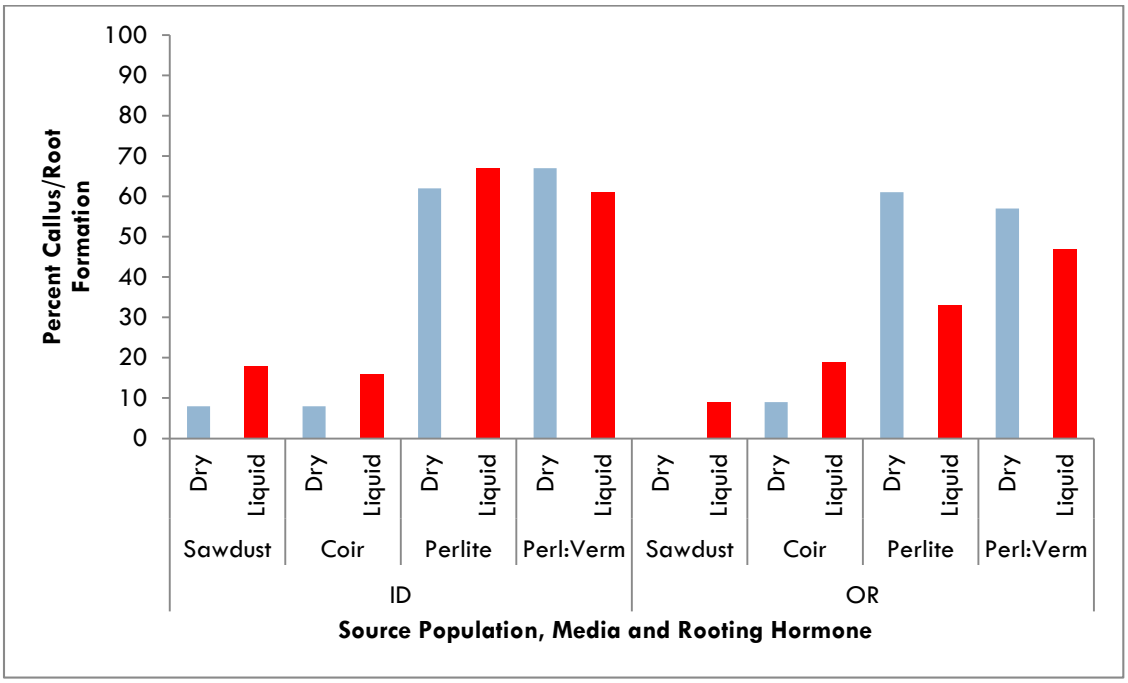

Figure 6. Percent callus and/or root formation for different media and root hormone combinations for Oregon and Idaho populations of *Rubus bartonianus*.

DISCUSSION

Preliminary results indicate that Bartonberry can be propagated from both seed and cuttings. Germination was relatively high for all treatments, including the untreated control. This would appear to indicate multiple viable options for managers looking to produce Bartonberry from seed. Continued monitoring of survival of seedlings from each of the treatments will allow us to further inform best practices.

Formation of callus and/or roots from Bartonberry cuttings varied among substrates. The perlite and perlite vermiculite mix had much higher formation than the coconut coir or the pine shavings. The cuttings were hand-watered, and the perlite mixes likely allowed better drainage and more consistent moisture management than the other materials. Continued monitoring of survival of cuttings from each treatment will continue to inform our recommendations.

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APPENDIX A. DETAILED PROTOCOLS FOR *RUBUS BARTONIANUS* SEED GERMINATION TRIALS.

Scarification

1. **Sulfuric Acid Treatment (550 seeds per replicate, USDA lab):**
   Materials: 5 test tubes, test tube holder, ice bath (Styrofoam), stirrer, gloves, safety goggles, lab coat, pipette, DI water, mouth guard, ice, 98% sulfuric acid, calcium hydroxide, calcium hypochlorite, cheese cloth, rubber bands, small wire strainer
   a. Contact Dr. Kim Hummer about use of the USDA lab. Dr. Sugae Wada taught us the protocol.
   b. Put on safety gear: double layer gloves, lab coat, goggles, mouth guard (when working directly with acid), eye protection
   c. Prepare ice bath in Styrofoam container, place on an absorbent towel, always keep hands dry
   d. Prepare neutralizing solution. Measure out 1.5 g of calcium hydroxide, put into 500 ml of DI water, stir until dissolved. Add 1.5 g of calcium hypochlorite, continue stirring on stir plate. Can use some heat to dissolve the material. Continue stirring until use.
   e. Dry hands well. Pour small amount of sulfuric acid (20 ml max) into small glass beaker. Wipe any residue that spills quickly and use a generous amount of water.
   f. Place 110 seeds into each test tube in the wire rack on ice. Note the time and calculate 30 minutes to know when you will remove the seeds from the acid. Fill 1st tube carefully, 1/3 full with sulfuric acid using a plastic pipette. Move slowly and take care not to drip acid on sides of tubes. Seeds will turn a black color.
   g. Every 5 minutes treat each of the remaining test tubes, use a glass stir rod to stir the others (once every 10 minutes).
   h. When there are 10 minutes remaining before taking out the first test tube, prepare the neutralizer by pouring it into 5 small jars. Prepare a rinse station with small pieces of cheese cloth, rubber bands, and a small wire strainer (tea strainer works well).
   i. To rinse seeds out of acid, pick up the test tube as low as possible. Run water at a medium rate. Hold the strainer in one hand, facing away from you. With the tube facing away, very quickly fill with water and simultaneously dump seeds into strainer, rinsing the tube quickly and allowing cold water to flow over seeds. If this happens to slowly the heat from the water and sulfuric acid can cook the seeds.
   j. Seeds will be blackened and charred. Under the running water, use your thumb to rub the black parts off. Have to use a lot of force. Once the seeds look clean (and brown, smooth), place in the jar with the neutralizer solution. Let it site in solution for 5 minutes. Cover jar with 2 layers of cheese cloth and a rubber band.
   k. Continue the rinsing process with each tube every 5 minutes (in the order they were treated so that all are treated for only 30 minutes). After each tube has been in the neutralizer for 5 minutes, place the cheese cloth directly up against the water faucet so that the solution is quickly rinsed out. The water will flow directly through the cloth into the

jar. Once there is no more white residue, place in a bucket with water dripping in for 30 minutes.

l. Remove jars from rinse bucket. Place seeds back into strainer and rub clean one more time.

m. If ready, plate seeds immediately. If not, seeds can be stored in DI water in a jar for 1-2 days prior to plating.

2. **Mechanical Scarification Treatment (550 seeds per replicate, OSU seed lab)**
    a. Talk to Dale Brown (OSU seed lab) about use of the seed scarifier and space to do bleach treatments.
    b. Test seeds with varying levels of manual sanding with sand paper to determine a good amount of scarification that will imbibe the seeds- these seeds are not part of experiment but are used for reference.
    c. To scarify, always do a test run using the small scarifier to make sure the timing is correct for the amount of sanding you want, using the seeds from your trials as a reference. Note the PSI that it runs (40 PSI in August 2014).
    d. Scarify 550 seeds. Place 110 seeds at a time in the small scarifier (Pneumatic seed scarifier, manufactured by Mater International). Run for 3 minutes for each round. Place seeds in envelope. Seeds should be sanded with a lot of their texture no longer present and are lighter in color than un-scarified seeds
    e. Place all seeds, once scarified, into a small beaker. Add enough bleach (100%) to cover the seeds. Note time the bleach has been added. Stir occasionally. Leave seeds in bleach for *15 minutes*.
    f. Rinse seeds using a strainer with distilled water, at least 3 times.
3. **Bleach treatment (550 seeds per replicate, OSU seed lab or IAE)**
    a. Place all seeds (550), into a small beaker. Add enough bleach (100%) to cover the seeds. Note time the bleach has been added. Stir occasionally. Leave seeds in bleach for *60 minutes*.
    b. Rinse seeds using a strainer with distilled water, at least 3 times.
4. **Control treatment (550 seeds per replicate)**

Plating in germination trays

1. Sterilize small square germination trays (6 per treatment) and forceps, rinse very well.
2. Label each tray with "RUBA, date, treatment (sulfuric, mechanical, bleach, or control), # of days in cold stratification (120, 90, or 60)"
3. Cut a piece of orange felt and blue blotter paper to fit the trays.
4. Dip the piece of felt very quickly in distilled water, place in germination tray.
5. Dip the blotter paper in distilled water and also and place on top of felt.
6. Place seeds in a 10 X 10 grid on the blotter paper, discard any broken seeds at this time. Mark on label the number of seeds present in each tray.
7. There will be approx. 50 seeds remaining, place on one final tray. Mark # of seeds present.

Stratification (OSU seed lab)

1. Place seeds in warm stratification for 30 days (20°C). Check on seeds once every week- spray with distilled water or 3% hydrogen peroxide solution if needed to abate fungal infection.
2. After 30 days, remove and place seeds into cold stratification for either 120, 90, or 60 days. All seeds will be ready for removal at the same date. Check on seeds weekly to make sure there are not fungus issues and that the blotting paper is moist. If needed, spray trays with a solution of 3% hydrogen peroxide + distilled water, or distilled water alone if tray is dry. Remove any seeds with notable fungus issues (fungus taking over the seed).
3. Note germination by keeping track of the date and location in the tray, with (0,0) being in the upper L corner of the tray for those in the 120 day treatment.