Improving Germination of Red Elm (*Ulmus rubra*), Gray Alder (*Alnus incana*) and Buffaloberry (*Shepherdia canadensis*) Seeds with Gibberellic Acid

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Abstract: Red elm (*Ulmus rubra*), gray alder (*Alnus incana*), and buffaloberry (*Shepherdia canadensis*) are considered important plants for many Native American tribes in the United States. Native Americans use these 3 species for a variety of traditional and medicinal purposes. For example, red elm is still the preferred firewood for the cultural ceremonies of several tribes. Kansas tribal leaders would like to plant more of these species on tribal land, but they have been difficult to germinate due to seed dormancy mechanisms. While red elm is valued as a ceremonial tree, it is susceptible to Dutch Elm Disease (*Ophiostoma ulmi*), and it is not widely grown in ornamental nurseries. This has led to declining natural tree populations and difficulties in commercial propagation. The objective of this study was to evaluate techniques to promote germination of red elm, gray alder, and buffaloberry seeds, with the long-term goal to improve the production of these plants commercially and enable tribes and land owners to increase the presence of these native plants on their lands. Studies were conducted with stratified and non-stratified red elm, gray alder, and buffaloberry seeds soaked in 1 of 4 treatments: 0, 250, 500 or 1000 ppm of gibberellic acid (GA3). Results indicate the use of gibberellic acid in high concentrations is effective with non-stratified seeds of red elm.

Keywords: Native American, propagation, cultural plants, medicinal plants

Introduction

Red elm (*Ulmus rubra*), also called slippery elm, is a native North American tree that is valued by many American Indian tribes as fuel for ceremonial fires at pow wows, funerals, or sweat lodges. Other past uses of red elm included the inner bark for cordage, fiber bags, and storage baskets. In spring, the cambium becomes very mucilaginous and has several medicinal uses including treatment for swollen glands, use for sore throats, and as an eyewash for sore eyes; women also drink a tea of the bark to make childbirth easier (USDA 2011a. Currently, tribes primarily use red elm for firewood in traditional ceremonies.

Gray alder (*Alnus incana*), also called tag alder, mountain alder, or hazel alder, is a species of moist lowlands, common in the region surrounding the Great Lakes including east-central Canada, Virginia, and Maryland. It is used locally for fuel and also supports symbiotic nitrogen-fixing bacteria in root nodules, which makes the alder valuable for improving soil fertility. Native Americans used alder to treat anemia, internal bleeding, urinary problems, bruises, backaches, and skin irritations (USDA 2011b). Alders are also used as landscape ornamentals.

Buffaloberry (*Shepherdia canadensis*) is also called soapberry, russet red buffaloberry, or Canadian buffaloberry. It is a native, deciduous, nitrogen-fixing shrub with broad distribution ranging from Alaska to Maine, south from New York to South Dakota, and south at higher elevations into Arizona. Buffaloberry fruits are eaten fresh or dried and also used to make "Indian ice cream". Berry juice is used to prevent heart attacks and indigestion. The berries are also chewed to induce childbirth (USDA 2011c).

Gibberellic acid (GA_3) is a naturally occurring plant hormone that can release seeds from dormancy. The positive effect of GA_3 promotes uniform seed germination and increases germination percentages (Adams et al., 2010). Gibberellic acid removes physiological dormancy mechanisms that often require lengthy stratification or light to maximize germination (Norden et al., 2007). Seeds of red elm, gray alder, and buffaloberry exhibit unknown dormancy issues and thus are the subject of this investigation. Specifically, this study evaluates the use of stratification and GA_3 to promote germination of red elm, gray alder, and buffaloberry seeds, with the long-term goal to improve the production of these plants commercially and enable tribes and land owners to increase the presence of these native plants on their lands.

Materials and Methods

Red elm (Ulmus rubra), gray alder (Alnus incana), and buffaloberry (*Shepherdia canadensis*) seeds were used for this study. In April 2010, red elm seeds were collected from 2 Kansas locations: Butler and Douglas Counties. Gray alder and buffaloberry seeds were obtained from Lawyers Nursery of Montana.

The experimental design for each species was a randomized complete block with a 2 × 4 arrangement of factorial treatments. The factorial design included 2 stratification treatments (no stratification or stratification) and 4 gibberellic acid (GA_3) treatments (0, 250, 500, 1000 ppm). The experiment was replicated 6 times using 2 petri dishes as a replication for each treatment, with 5 seeds per petri dish. Prior to stratification or GA_3 treatments, buffaloberry seeds were scarified using sulfuric acid (SO_4) for 20 minutes and rinsed with tap water.

All seeds were divided into 2 stratification treatments, air-dried for 3 days, and stored in sealed plastic containers. The stratification treatment started in May, with seeds received cool, moist stratification at 5 °C (41 °F) for 90 days. Stratification was accomplished by placing 60 seeds per pouch (fabric bag) in 1 polyethylene bag containing 454 g (1 lb) of moist germination media (peat moss). The remaining 240 seeds were immediately treated with GA_3 (Research Organics, Cleveland, OH) at 0, 250, 500, 1000 ppm. For each GA_3 treatment, sixty seeds were placed in beakers containing 120 ml (4 oz) of GA_3 solution and placed on a shaker at 175 revolutions per minute for 24 hours. Seeds were then placed on moist filter paper inside 47 mm (1.9 in) diameter petri dishes (Fisher Scientific, USA) using 2 ml (0.07 oz) of distilled water to maintain humidity. Petri dishes were placed on a lab bench at room temperature 18°C (65 °F) until seed radicle emergence. Stratified seeds were handled identically upon removal from the germination media after 90 days.

Germination was monitored every 3 days for all the seeds and recorded when emerged radicles reached a length of 3 mm (0.1 in). Data was collected over a period of at least 2 weeks, after germination began, and ended when no additional seeds germinated for 6 days. Data was subjected to ANOVA (Statistical Analysis System, SAS Institute Inc., Cary, NC) and the means separated by LSD test ($p<0.05$).

Results and Discussion

Red elm

Both Douglas and Butler County red elm seedlots exhibited similar trends in germination across the various treatments (Table 1); however, statistical analysis revealed a strong interaction between the stratification and GA_3 treatments. In general, non-stratified seeds showed a positive relationship between GA_3 concentration and germination; for example, as concentrations of GA_3 increased, so did germination percentage. Conversely, stratified seeds exhibited a negative relationship between GA_3 concentration and germination; stratified seeds that received increasing concentrations of GA_3 decreased in germination percentage.

For Douglas County red elm seeds, the highest germination occurred in the non-stratified, 1000 ppm GA_3 treatment. The stratified, 1000 ppm GA_3 treatment resulted in the lowest germination percentage (Table 1). For the Butler County seedlot, both the 500 and 1000 ppm GA_3, non-stratified seeds performed similarly (Table 1); this treatment combination also yielded the highest germination percentages. Similar to the Douglas County seedlot, stratified seed exposed to 1000 ppm GA_3 had the poorest germination.

Germination of non-stratified red elm seeds was maximized with GA_3 at 1000 ppm. Previous work has indicated that stratification should increase seed germination (Dirr and Heuser, 2006). While this was true for the control treatment not exposed to GA_3, 90-day stratified seeds that received GA_3 resulted in significantly less germination than non-stratified seeds. Thus, the GA_3 treatment exhibited a negative effect on germination of stratified seeds. This was shown by the strong interaction between the stratification and GA_3 treatments.

Table 1. Germination percentage of red elm (*Ulmus rubra*), gray alder (*Alnus incana*), and buffaloberry (*Shepherdia canadensis*) after gibberellic acid (GA_3) and stratification treatments.

GA_3 concentration (ppm)	*Ulmus rubra*, Douglas County†		*Ulmus rubra*, Butler County†		*Alnus incana*‡		*Sheperdia canadensis*‡	
	Non-stratified	Stratified	Non-stratified	Stratified	Non-stratified	Stratified	Non-stratified	Stratified
0	13.3 c B	30.8 a A	30.0 b B	47.3 a A	3.3	8.3	5.0	6.6
250	28.3 bc A	22.3 ab A	50.0 b A	30.5 b B	8.3	5.0	5.0	0
500	45.0 b A	14.1 b B	73.3 a A	19.5 bc B	3.3	1.6	1.6	3.3
1000	78.3 a A	5.6 c B	86.6 a A	2.8 c B	10.0	3.3	1.6	0

† Within a county, means within a column (lower case) or row (uppercase) followed by the same letter were not significantly different (LSD $p < 0.05$; n = 6).

‡ No significant difference (LSD $p < 0.05$; n = 6).

One explanation for this may be a supraoptimal response of stratified red elm seeds to treatment with GA_3. Non-stratified seeds benefited from exposure to exogenous GA_3 GA_3, while stratified seeds (which naturally produce endogenous GA_3 experienced an apparently toxic or inhibitory response when exposed to additional GA_3. This relationship will be an important one to investigate in the future.

Gray alder

It has been suggested that gray alder seeds can benefit from 60 to 90 day stratification (Schalin 1967; USDA 2011a), but this study found no significant differences among either stratification or GA_3 treatments. Unlike red elm, gray alder experienced overall poor germination with both stratification and GA_3 treatments (Table 1). Results suggest that further work is needed to improve seed propagation of gray alder; under these treatment conditions, seed propagation may not be a viable option for mass production of this species.

Buffaloberry

Similar to gray alder, no significant differences were detected between stratification and GA_3 treatments. Overall, germination was very low among all treatment combinations < 7% (Table 1). A previous study (Dirr and Heuser, 1987) recommends scarification for 20 to 30 minutes followed by a period of 2 to 3 months stratification. Other authors (Krishnan et al. 1991) recommend that buffaloberry be rooted by cuttings due to the low viability of the seeds.

Summary

Based on this study, we recommend non-stratified red elm seeds to be soaked in 1000 ppm GA_3 for 24 hours before sowing. Seeds typically took 10-15 days to germinate. Future studies should evaluate higher levels of GA_3 and shorter stratification periods to determine optimum rates for maximum germination. A study using higher concentration of GA_3 (500, 1000, 2000, 4000 ppm) is currently being conducted to determine the upper limit to improve germination. We do not have seed treatment recommendations for gray alder and buffaloberry due to the poor germination displayed in this study. Additional studies will examine rooting capacity with dormant and greenwood cuttings of the 3 species.

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