The influence of light and a smoke-derived compound on the germination requirement of Wyoming Big Sagebrush (*Artemisia tridentata* spp. *wyomingensis)* in the Great Basin.

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Introduction

Once occupying 150 million acres, the Sagebrush-Steppe ecosystem has declined by 90%, making it one of the most imperiled systems in the United States[9]. This decline has been driven by invasive exotic species, overgrazing and agricultural overuse, rapid land development for energy resources, and increased incidence of catastrophic wildfires[1]. The resulting decline of important native plant species such as sagebrush (*Artemisia tridentata*) in this area has destabilized the ecosystem structure and function and caused a loss of habitat for a diversity of organisms (e.g. Greater Sage Grouse, pigmy rabbit, etc.)[2, 3]. This ecosystem decline necessitates large-scale restoration, which can be achieved through direct seeding, as the use of plugs is prohibitively costly and labor intensive. Low seed germination can be a key limiting factor to plant reestablishment[4], but restoration outcomes can be improved through understanding species-specific germination requirements (such as the role of light [photoblastism]). Furthermore, seed enhancement technologies (e. g. seed pelleting and coating) have the potential to further improve plant establishment from seed, but require an understanding of the photoblastic requirements of sagebrush seed.

Objectives

Seeds of *A. tridentata* ssp. *wyomingensis* were used in two experiments to (1) determine the role of light in germination and to (2) identify whether and in what dosage can KAR_1 that can be used to overcome the light-induced germination barrier if one is present. It is hypothesized that this compound may overcome the light requirement and stimulate germination in such cases.

	(1) Photoblastic	(2) Dosage trial
Substrate (0.75% agar)	DI H_2O or KAR_1 (0.1µM)	KAR_1 (0, 0.1, 0.5, 1.0, 5.0, 10.0 µM)
Light Treatment (12 hr light: 12 hr dark, or 24 hr dark)	Light or Dark	Dark
Temperature ˚C (12hr day: 12 hr night)	15/5, 20/10, 25/15	15/5, 20/10, 25/15

Figure 1: The experimental design for germination trials of both photoblastic and dosage studies. Each combination of conditions consisted of four replicates of 25 seeds.

Methods

(1) The first trial examined the germination response to the presence or absence of light and KAR_1 across a range of diurnal temperature conditions for 28 days.

(2) The second trial examined the effect of KAR_1 dose response on seed germination in the dark within 14 days.

All seeds were sterilized in a 1% sodium hypochlorite solution for thirty minutes, double-rinsed with DI water, plated onto 60 mm Petri dishes under a laminar flow hood, and sealed with Parafilm. Germination was scored weekly for the light treatments and once at the end of the study period for both light and dark. A cut/squeeze test was performed to assess viability of ungerminated seeds at the final scoring. (See Figure 1 for full design).

(1) Data were checked for normality and homogeneity of variance and analyzed using a 3-way ANOVA7 (dplyr, vegan, agricolae, and car packages)[7].

(2) A two-way ANOVA was used to assess the substrate effect with temperature interaction on cumulative germination percentage after 14 days for dosage trials.

Pair-wise comparisons of treatment differences were estimated using Tukey Honest Significant Difference (HSD) post-hoc test and were considered significant at an $\alpha = 0.05$.

Image 1: (top left) *Artemisia tridentata* ssp. *wyomingensis.* Source: Matt Lavin. Image 2: (top right) Greater Sage grouse in a lek mating area. Source: Jeannie Stafford.

Results

(1) All treatments had a significant effect on seed germination. There was a significant interaction between substrate treatment ($p = 0.029$), temperature ($p = 0.004$), and light/dark ($p < 0.000$) on the overall germination percentage ($p < 0.000$, $F = 31.905$, $df = 11$). For dark conditions, samples treated with KAR_1 were found to have germination rates that were on average 11%, 15%, and 29% higher than the corresponding water treatments (Figure 2). Pair-wise comparison revealed that only the 25/15 °C temperature samples were found to significantly differ in germination percentage under dark conditions as a consequence of substrate. Germination in light conditions was not heavily impacted by treatment with KAR_1 as germination is near 100% in light conditions at all temperatures. (2) Dosage trials yielded a significant interaction between KAR_1 concentration ($p = 0.05221$) and temperature ($p < 0.000$) on germination percentages in the dark ($p < 0.000.$, $F = 20.198$, $df = 5$)(Figure 3).

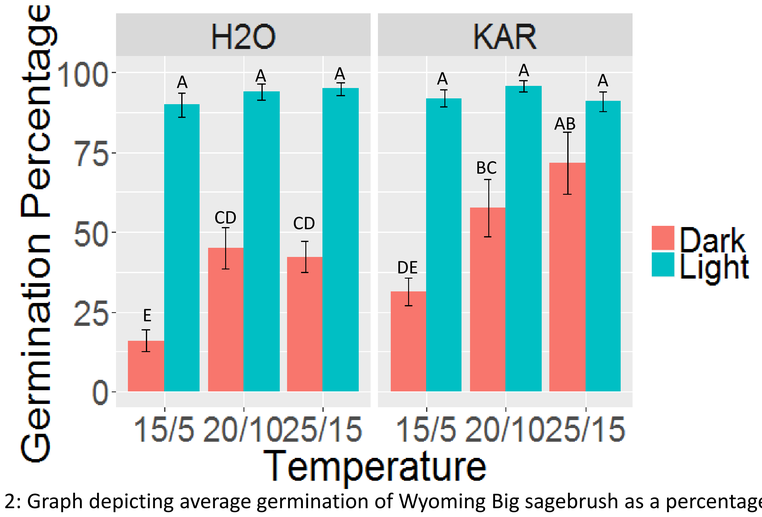

Fig. 2: Graph depicting average germination of Wyoming Big sagebrush as a percentage after a 28-d testing period. Data are grouped by dark (pink) or light (blue) conditions, temperature, and substrate content. Each bar represents the mean of four 25-seed replicates.

Discussion/Conclusions

Wyoming Big sagebrush exhibits positive photoblastism under normal germination conditions, requiring light to germinate. This is likely to inhibit germination in the field (especially at lower temperatures) if seeds are buried or if seed enhancement techniques used exclude light. Successfully reestablishing Wyoming Big sagebrush is critical to the overall health of a native plant community and in turn important to the recovery of an ecosystem following disturbances in the Great Basin[3]. Taken together, the results of two trials suggest that the germination inhibition due to dark conditions is lowest at higher temperatures (25/15 °C). Although there is a significant effect of KAR_1 at higher temperatures in the dark (photoblastic), results of the dosage trial show no significant effect of KAR_1 dosage at higher temperatures compared to the control. Thus, light exposure of seeds is critical for germination. Further research should quantify the impact of seed-technologies (e.g. pelleting and coating) on light uptake by Wyoming Big sagebrush seeds as well as evaluate the effectiveness of other compounds (e.g. smoke water) to overcome photoblastism in this species.

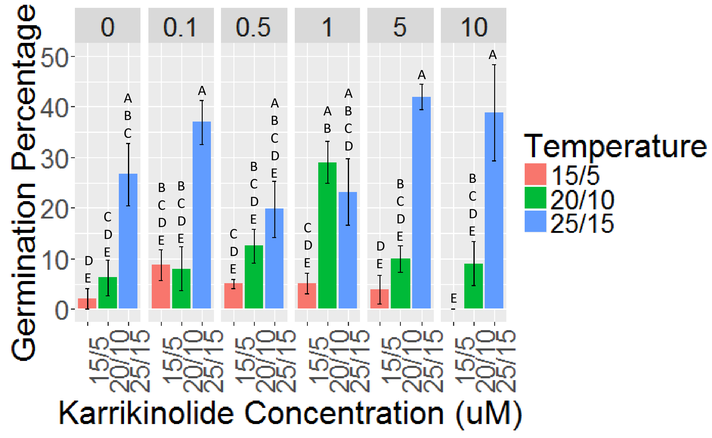

Fig. 3: Average germination as a percentage of Wyoming Big sagebrush after 14 days. Data are grouped by temperature conditions, and KAR_1 concentration. Each bar represents a mean of four 25-seed replicates.

References

[1] Connelly, J. W., Knick, S. T., et al. (2011). *Conservation of greater sage-grouse- a synthesis of current trends and future management*. Pp. 549–563 in S. T. Knick and J. W. Connelly (editors). Greater Sage-Grouse: ecology and conservation of a landscape species and habitats. Studies in Avian Biology (vol. 38), University of California Press, Berkeley, CA.

[2] Dumroese, R. K., Luna, et al. (2015). Conserving and restoring habitat for Greater Sage-Grouse and other sagebrush- obligate wildlife: the crucial link of forbs and sagebrush diversity. *Native Plants Journal, 16*(3), 276-299.

[3] Huber-sannwald, E., & Pyke, D. A. (2005). Establishing Native Grasses in a Big Sagebrush–Dominated Site: An Intermediate Restoration Step. *Restoration Ecology, 13*(2), 292-301.

[4] James, J. J., & Svejcar, T. (2010). Limitations to Postfire Seedling Establishment: The Role of Seeding Technology, Water Availability, and Invasive Plant Abundance. *Rangeland Ecology & Management, 63*(4), 491-495.

[5] Madsen, M. D., Davies, K. W., Williams, C. J., & Svejcar, T. J. (2012). Agglomerating seeds to enhance native seedling emergence and growth. *Journal of Applied Ecology, 49*(2), 431-438.

[6] Nelson, D. C., Risborough, J.-A., Flematti, et al. (2009). Karrikins Discovered in Smoke Trigger Arabidopsis Seed Germination by a Mechanism Requiring Gibberellic Acid Synthesis and Light. *Plant Physiology, 149*(2), 863-873.

[7] RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

[8] Scaffidi, A., Flematti, G. R., Nelson, et al. (2011). The synthesis and biological evaluation of labelled karrikinolides for the elucidation of the mode of action of the seed germination stimulant. *Tetrahedron, 67*(1), 152-157.

[9] Thompson, Jonathan. 2007. Sagebrush in western North America: habitats and species in jeopardy. Science Findings 91. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 5 p

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