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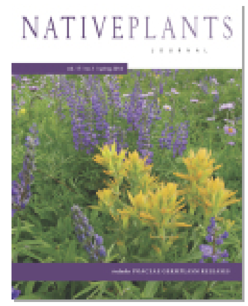

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ABSTRACT

As the capability of land management agencies to restore degraded habitat at large scales has improved, the availability of native plant materials has become a primary limiting factor in the restoration process. Developing clear protocols for a suite of regionally important restoration species will increase the feasibility and cost effectiveness of native species production on a commercial scale. A full factorial design was used to test a 1:100 smoke-water imbibe treatment coupled with 6 lengths of cold-moist stratification (0, 15, 30, 60, 90, and 120 d) to determine if 10 selected South Puget Sound prairie species have a dormancy that is broken by some combination of these factors. Plant-derived smoke-water is a proven germination cue in other fire-adapted ecosystems; however, smoke-water had a significant influence for only one—*Aquilegia formosa* Fisch. ex DC. (western columbine; Ranunculaceae)—of the 10 tested species after the 90-d stratification period. The duration of cold-moist stratification time had a significant effect on 8 of the analyzed species. Of those 8 species, 7 reached a maximum germination rate before the standard protocol guideline of 84-d cold-moist stratification, suggesting that stratification for species with unknown germination requirements may need to be shortened. Stratification times identified here will provide guidance and will improve production efficiency for producers interested in propagating these prairie species for restoration.

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KEY WORDS

restoration, propagation, forbs, seed pre-treatment, imbibe, pre-chill, Asteraceae, Caryophyllaceae, Fagaceae, Poaceae, Primulaceae, Ranunculaceae, Scrophulariaceae

NOMENCLATURE

USDA NRCS (2015)

Photos by Rod Gilbert

Prairie-oak ecosystems are among the most endangered systems in the US (Floberg and others 2004) because of fire exclusion, invasive exotic species, encroachment of native trees, and conversion to agriculture and other land uses. Only 3% of historic South Puget Sound (Washington, USA) grassland soils are still dominated by native prairie vegetation (Crawford and Hall 1997). Large-scale (hundreds to thousands of acres) restoration efforts to re-establish the native flora and fauna are currently underway in the South Puget Sound region and in other locations within the Willamette Valley–Puget Trough–Georgia Basin Ecoregion (Dunwiddie and Bakker 2011). Stanley and others (2011) found that because of insufficient seedbanks, restoring invaded prairies with mowing, herbicide, and prescribed fire treatment combinations were effective only if native seeds were also added. Having sufficient quantities of native seeds requires large-scale production of genetically diverse plants to increase community resilience (Drake and others 1998). As the capacity for land management agencies to work at these larger scales has improved through additional personnel, knowledge, funding, and (or) land acquisitions, the availability of native plant materials has become the primary limiting factor in the restoration process. Most of the native annual and perennial grasses and forbs used in South Puget Sound prairie restoration are not available for purchase from horticultural suppliers. Therefore, regional native seed farms are responsible for producing these propagules for restoration purposes.

Regionally and genetically appropriate seeds are limited in supply, and thus, maximizing germination is critical. To date, detailed propagation protocols for many native prairie plant species have not yet been developed, nor published, and many species do not respond well to standard propagation methods. We selected 10 of these challenging species that represent various taxonomic groups and examined how different seed pretreatment methods might improve germination rates.

Plant-Derived Smoke-Water

Fires started by Native Americans repeatedly swept through South Puget Sound prairies for thousands of years, with return intervals ranging from 1 to 5 y (Storm and Shebitz 2006). Many plant species respond well to fire, which is demonstrated by either increased germination and establishment immediately post-burn (Maret and Wilson 2000) or by enhanced growth and reproductive capacity in the years following fire (Pyke and others 2010). Potential reasons for enhanced germination and establishment post-burn include, for example, physical scarification of the seeds from fire, also known as "heat shock" (Bell and others 1993; Baskin and Baskin 1998; Williams and others 2003), and enhanced access to resources (light, moisture, and nutrients) post-burn, or "competitive release" (Jutila and Grace 2002). Fire-adapted plant species, however, have historically been difficult to germinate in the greenhouse setting using conventional propagation protocols (Landis 2000; Van Staden and others 2000). Research has shown that these species may be lacking some fire-related germination cues. Plant-derived smoke-water has been shown to increase germination rates for a wide range of species in fire-adapted ecosystems such as the fynbos of South Africa, the kwongan of western Australia (Van Staden and others 2000), the chaparral of California (Keeley and Fotheringham 1998), and grasslands, shrublands, and forests of the interior western US (Fornwalt 2015). Incomplete combustion of organic materials produces water-soluble compounds broadly classified as butenolides (Flematti and others 2004; Jefferson and others 2014), which have been shown to improve germination rates in more than 60 species across 26 different plant families (Chiwocha and others 2009) and across seed dormancy classes (Baskin and Baskin 1998). Smoke-water may act as both a chemical scarification of the seedcoat (Keeley and Fotheringham 1998) and a physiological cue for seed response to other growth hormones (Strydom and others 1996). Currently, extensive primary research is proceeding on the exact chemical compounds and multiple physiological pathways responsible for breaking seed dormancy and increasing plant vigor. In the meantime, it may be most pragmatic for native producers to take an applied research approach for species of restoration interest. Determining whether certain smoke-water treatments result in a higher germination rate (relative to the standard

Two species in our experiment: large, showy deltoid balsamroot (*Balsamorhiza deltoidea*) and smaller western buttercup (*Ranunculus occidentalis*).

Flower of western columbine (*Aquilegia formosa*), another species tested in our study.

of de-ionized water) for any of the tested species could provide improvements to nursery protocols that could result in higher productivity.

Seed Stratification

Many South Puget Sound prairie species exhibit physiological dormancy, a type of dormancy that prevents germination until chemical or environmental changes occur. These species typically respond positively to cold-moist stratification pretreatments of seeds (Drake and others 1998). Summer- and fall-dispersed species require cold (0–5 °C [32–41 °F]) stratification after initial uptake of water (imbibition) and must be kept moist until germination (Baskin and Baskin 1998). Continued stratification of seeds after they have germinated may promote pathogen growth and can complicate sowing due to the presence of radicles. Some species are sensitive to desiccation after emergence of the radicle (Senaratna and McKersie 1983), therefore increasing the likelihood of seed death during handling. Because of these factors, seeds that germinate while still under stratification conditions are rendered unusable. Researchers have suggested that a stratification period of 12 wk (84 d) be used for species that are likely to require stratification but for which there has been no species-specific protocol developed (Baskin and Baskin 1998). Select Puget lowland prairie species have shown optimal cold-moist stratification periods varying from 0 to 12 wk (Drake and others 1998; Russell 2011; Jones and Kaye 2015). See Table 1 for a summary of conspecific germination results. Identifying the shortest stratification period necessary to overcome dormancy, while preventing early germination and seed damage, will increase efficiency and efficacy of native plant production.

METHODS

Species Selection

We chose 10 species for this experiment based on their importance in prairie restoration and the availability of appropriate seed stock (Table 2). We also made an effort to cover a wide range of taxonomic groups and life histories of plants desirable for prairie restoration in the South Puget Sound region. All species tested had at least one published protocol, but these often reported very low germination rates (Table 1), so we saw an opportunity to potentially improve on those numbers. Seeds were wild collected from South Puget Sound prairies or collected from a local native seed farm intended to increase regional seed availability. Farm-collected seeds had been wild collected within the past 2 y and grown for one generation. All seeds were cleaned using our standard cleaning procedures and stored under the same conditions (40 °C [104 °F] and 40% humidity) in a cold storage facility from between 5 mo and 4 y before use in this project. Older seeds may have experienced greater variability in storage conditions. For species that had seeds collected from more than one site and (or) date (different seedlots), seeds were thoroughly mixed prior to sowing to ensure even distribution of seed source among treatments.

Experimental Design

We used a full factorial design to test the smoke-water imbibe treatment coupled with a range of lengths of cold-moist stratification to determine if any of our 10 target species have a dormancy that is broken by some combination of these factors. There were 2 imbibe treatments: de-ionized water and smoke-water solution of 1:100, in addition to the 6 cold-moist stratification durations: 0, 15, 30, 60, 90, and 120 d. Each treatment had 5 replicates of 50 seeds. A total of 3000 seeds were used for each species.

Smoke-Water Imbibition

The first factor, smoke-water imbibition, tested the effects of plant-derived smoke-water on germination as compared to standard de-ionized water. Seeds were counted and placed in Petri plates lined with 4 mm filter paper. Smoke-water was made based on the protocol of de Lange and Boucher (1990), which consisted of a 2-part process. 1) A combustion chamber (20 l [5.3 gal]) burned dry plant chaff from *Festuca roemeri* (Pavlick) Alexeev (Roemer's fescue; Poaceae), *Collinsia parviflora* Lindl. (maiden blue eyed mary; Scrophulariaceae), and *Eriophyllum lanatum* (Pursh) Forbes (common woolly sunflower; Asteraceae) (3 kg [6.6 lb]) over coals of native *Quercus garryana* Douglas ex Hook. (Oregon white oak; Fagaceae) (~4 kg [8.8 lb]), and air-flow regulation resulted in a slow, cool burn (130–170 °C [266–338 °F] internal chamber temperature). 2) The smoke flowed by convection into a cooling chamber (60 l [15.9 gal]) and was then drawn through 5 l (1.3 gal) of de-ionized water in an aspirator bottle for 1 h by vacuum suction. The dark-brown solution (termed plant-derived smoke-water) was stored in a laboratory freezer (–12 °C [53.6 °F]) until use.

We administered the same batch of smoke-water for all replicates. A 1:100 smoke-water dilution was prepared and approximately 3 ml (0.1 oz) of solution was pipetted into each smoke-water replicate, while 3 ml (0.1 oz) of de-ionized water was pipetted into the control replicates. Replicates of each species were imbibed for prescribed lengths of time (8, 12, or 24 h) until the water uptake of the seed plateaued (Smith and others 2013). After imbibition, all replicates were rinsed with de-ionized water to mimic rainfall, covered to retain 100% relative humidity, and placed in a germination chamber (Hoffman Manufacturing Inc, SG30 Controlled Environment Chamber, Jefferson, Oregon) at 3 °C (37.4 °F) and 24 h dark to begin stratification.

Cold-Moist Stratification

The second factor, stratification length, tested germination responses to 0, 15, 30, 60, 90, and 120 d of cold-moist stratification.

TABLE 1

Recommended stratification time and resulting average percentage of germination.

Species	Stratification treatment	Percentage of germination (%)	Source
Aquilegia formosa	**60 d cold-moist**	**74**	**This study's recommendation**
	62 d warm-moist	0	Russell 2011
	62 d cold-moist	4	Russell 2011
	3 d cold-moist	8	*Trindle and Flessner 2003*
	180 d cold-moist	15	*Trindle and Flessner 2003*
Balsamorhiza deltoidea	**30 d cold-moist**	**28**	**This study's recommendation**
	42 d cold-moist	31	Drake and Ewing 1998
Castilleja levisecta	**30 d cold-moist**	**65**	**This study's recommendation**
	56–112 d, 3rd-y seed	0	Wentworth 1994
	56–112 d, 2nd-y seed	13	Wentworth 1994
	42–56 d cold-moist	20–95	Lawrence 2005
	56–112 d, 1st-y seed	47	Wentworth 1994
	42 d cold-moist	> 75	Kaye and Lawrence 2003
	42 d cold-moist	80	Caplow 2004
Dodecatheon hendersonii	**15 d cold-moist**	**70**	**This study's recommendation**
	84 d cold-moist	59	Drake and Ewing 1998
Dodecatheon pulchellum	**60 d cold-moist**	**91**	**This study's recommendation**
	0 d	0	*Skinner 2006*
	30 d cold-moist	0	*Skinner 2006*
	42 d warm, 42 d cold	0	Guerrant and Raven 1998
	42 d cold-moist	22	Guerrant and Raven 1998
	42 d cold-moist	44	Drake and Ewing 1998
	0 day	48	Guerrant and Raven 1998
	Frozen and 0 d	59	Guerrant and Raven 1998
	150 d cold-moist	65	*Skinner 2006*
	150 d cold-moist	65	*Evans and others 2008*
Gaillardia aristata	**90 d cold-moist**	**46**	**This study's recommendation**
	0 d	8	*Skinner 2006(b)*
	0 d	61	Roemmich 2011
	28 d cold-moist	76	Byers and others 2004
	14 d cold-moist	80	Roemmich 2011
	30 d cold-moist	92	*Wick and others 2008*
Ranunculus occidentalis	**0 d**	**62**	**This study's recommendation**
	Frozen and 0 d	18	Guerrant and Raven 1998
	0 d	21	Guerrant and Raven 1998
	42 d cold-moist	52	Drake and Ewing 1998
	42 d cold-moist	87	Guerrant and Raven 1998
	42 d warm, 42 d cold	91	Guerrant and Raven 1998
Silene douglasii	**60 d cold-moist**	**76**	**This study's recommendation**
	70 d cold-moist	23	Vance 2010

Notes: Our recommended stratification time and resulting average percentage of germination using de-ionized water are listed first, in bold, for each species. A selection of other sources is listed in order of increasing percentage of germination for comparison. Sources in italics are found on the Native Plant Network website.

TABLE 2

Species selected for germination trials and their corresponding collection date, source location, and geographic origins.

Species	Common name	Family	General distribution*	Date(s) collected	Source of collection	Origins**
Aquilegia formosa Fisch. ex DC.	western columbine	Ranunculaceae	western US	10/1/2011	Wild	JBLM
Balsamorhiza deltoidea Nutt.	deltoid balsamroot	Asteraceae	WA, OR, and CA	10/1/2011 7/9/2011	Wild and Nursery	Unknown; GH
Carex inops L.H. Bailey	long-stolon sedge	Cyperaceae	western and central US	7/20/2010 8/5/2010 12/31/2009	Wild and Nursery	MM; SPS prairies
Castilleja levisecta Greenm.	golden Indian paintbrush	Scrophulariaceae	western WA and western OR	12/31/2008	Wild	Rocky Prairie
Dodecatheon hendersonii A. Gray	mosquito bills	Primulaceae	western WA, western OR, and CA	6/21/2010	Wild	JBLM
Dodecatheon pulchellum (Raf.) Merr.	darkthroat shootingstar	Primulaceae	western and central US	7/6/2011	Wild	JBLM
Gaillardia aristata Pursh	blanketflower	Asteraceae	western, north-central, and northeastern US	10/1/2010 7/29/2010	Nursery	JBLM
Ranunculus occidentalis Nutt.	western buttercup	Ranunculaceae	western US	6/13/2012	Farm	Unknown
Silene douglasii Hook.	Douglas's catchfly	Caryophyllaceae	western US	8/5/2011	Nursery	JBLM
Solidago missouriensis Nutt.	Missouri goldenrod	Asteraceae	western, central, and eastern US	10/1/2011	Nursery	Glacial Heritage

Notes: Different collection dates represent different seedlots.

* From USDA PLANTS database.

** JBLM = Joint Base Lewis McChord; MM = Mima Mounds Natural History Area; GH = Glacial Heritage Natural Preserve; SPS prairies = across South Puget Sound prairie sites.

All replicates were kept in a germination chamber at 3 °C (37.4 °F), were monitored twice weekly for pathogens and adequate moisture, and were randomly repositioned. Seeds that germinated during stratification, prior to the initiation of the germination sequence, were considered "unusable" because the presence of radicles inhibits proper sowing techniques. These seeds were counted and recorded separately and are reported with hatch marks in our results (Figure 1). Following the prescribed number of stratification days, replicates were submitted to a germination sequence of light and temperature fluctuations. A 12-h photoperiod with diurnally alternating temperatures of 15 °C and 6 °C (59 °F and 42.8 °F) was used. This sequence mimicked spring conditions typical of South Puget Sound prairies. Seeds were counted as "germinants" upon emergence of a 2 mm (0.08 in) radicle and were then removed from the Petri plate. All replicates were monitored for germination for 6 wk after stratification was complete.

Data Analysis

Percent germination data for each species were analyzed using a two-way ANOVA to evaluate effects of stratification time, smoke-water treatment, and their interaction on germination rate. All data were arcsine square root transformed prior to analysis to achieve homogeneity of variance. Post-hoc comparisons between treatments were evaluated using Tukey's HSD. An alpha of 0.05 was used to determine significance, and $N = 5$ for all comparisons. All analyses were conducted using StatPlus:mac 5.8.2 (AnalystSoft 2009, Walnut, California).

RESULTS

Sufficient germination occurred to evaluate treatment effects for 8 out of 10 species. *Carex inops* L.H. Bailey and *Solidago missouriensis* Nutt. were excluded from analysis because of very low maximum germination rates, < 2 and < 5% respectively, for

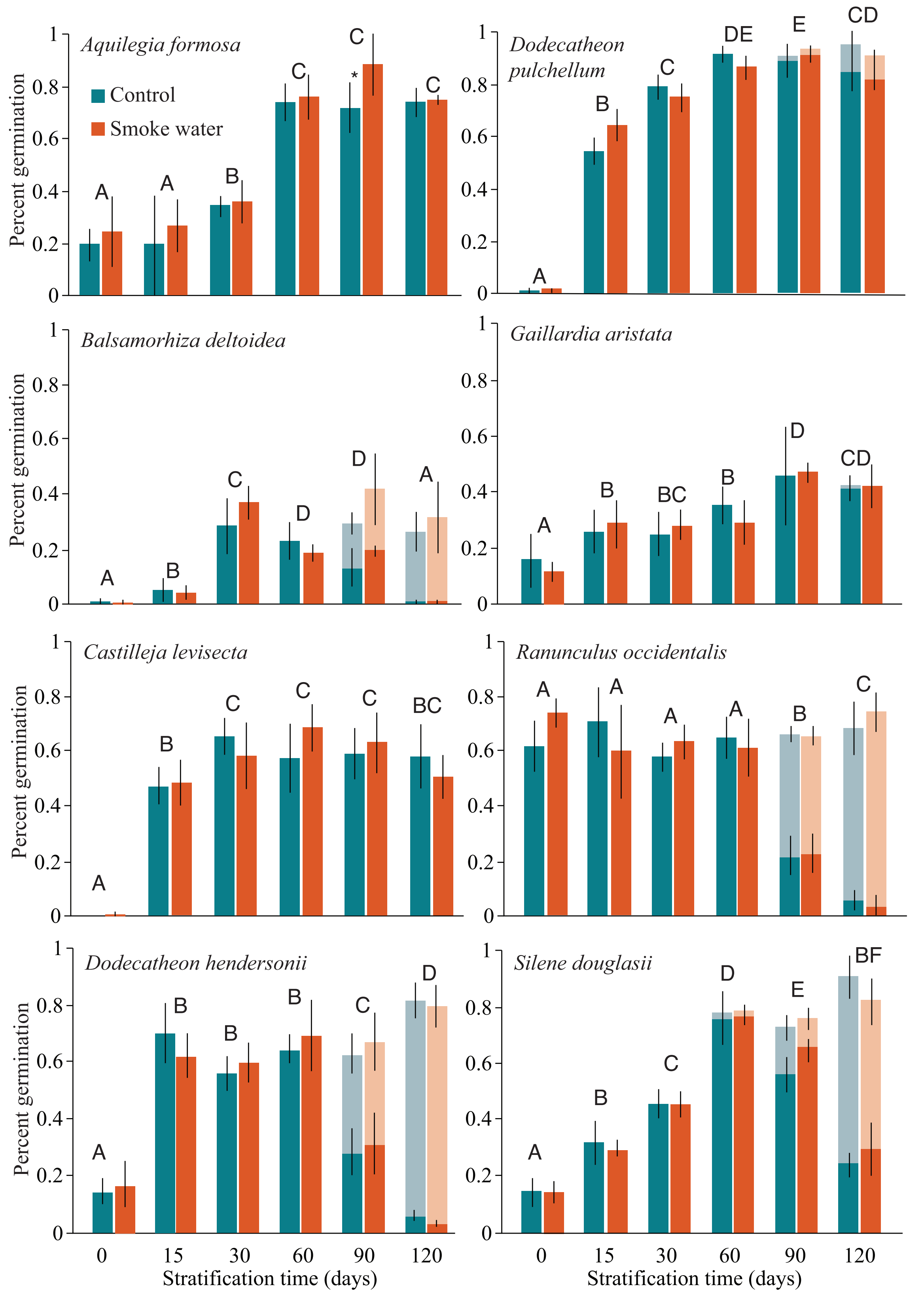

Figure 1. Effects of stratification time and imbibe treatment on germination of 8 prairie species. Light blue- and peach-colored columns represent proportion of seeds that germinated early (before the end of the assigned stratification time), rendering them unusable. Teal- and orange-colored columns represent percent germination post-stratification. Average percentage of germination for each treatment ($n = 5$) ± 1 SD is shown. Asterisks represent significant differences ($P < 0.05$) between smoke-water and control treatments; different letters represent significant differences ($P < 0.05$) in percent germination post-stratification.

any treatment combination. Therefore, germination requirements for *Carex inops* and *Solidago missouriensis* are not presented here.

Smoke-Water

Smoke-water imbibition treatment significantly increased the germination rate of *Aquilegia formosa* after 90 d in stratification. For this stratification time, smoke-water increased *A. formosa* average germination from 72 to 89% ($P < 0.03$; see Figure 1). Other species responded no differently to smoke-water than they did to the control after any stratification time tested.

Stratification

The duration of cold-moist stratification time had a significant effect on the percent germination of all 8 of the analyzed species ($P < 0.01$ for all species; see Figure 1). The length of time needed to break physiological dormancy with cold-moist stratification was species-specific. If 2 or more treatments produced similar germination rates, we incorporated the shorter duration into protocols (Table 1) as it increased production efficiency and minimized the likelihood of damage from pathogens in stratification. *Ranunculus occidentalis* had relatively high germination rates across all stratification treatments (58–75% average), thus stratification time may not be useful in increasing the propagation efficiency of this species. *Dodecatheon hendersonii* responded well to at least 15 d of cold-moist stratification. *Balsamorhiza deltoidea*, a notable pollinator resource, and *Castilleja levisecta*, a federally threatened species, both benefited from a 30-d cold-moist stratification. These results could aid restoration and reintroduction efforts of these important species. *Dodecatheon pulchellum* and *Silene douglasii* benefited from a 60-d stratification, while *Gaillardia aristata* benefited most from a 90-d stratification time.

Balsamorhiza deltoidea, Dodecatheon hendersonii, Ranunculus occidentalis, and *Silene douglasii* had a relatively high proportion of seeds that germinated during the stratification period (typically 90- and 120-d treatments). These seeds were considered "unusable" because the presence of radicles inhibits proper sowing techniques, and they may be at increased risk of desiccation and damage from fungal and bacterial growth (see Figure 1).

DISCUSSION

In the South Puget Sound, availability of native plant materials has become the primary limiting factor in the prairie restoration process, making efficient production of plant material vital. Of the 10 recalcitrant species selected for this study, 8 exhibited species-specific responses to the smoke-water and cold-moist stratification treatments, while 2 had no measurable response. Identifying specific germination cues and optimizing stratification time will further increase efficiency of plant propagation.

Carex inops and *Solidago missouriensis* had virtually no germination with any of our treatments. We suspect that the poor germination of *S. missouriensis* was attributable to inherently low viability. Another tested seedlot of this species revealed only 4.1% pure live seed (Hamman and others 2014). Researchers have suggested that *C. inops* may need a second period of stratification for good germination (Sheehan 2013), or, like many other *Carex* species, may reproduce primarily by rhizomes or stolons (Bernard 1990). Further research is needed on seed stratification requirements of these and other recalcitrant prairie species.

Smoke-Water

Smoke-water treatment was a successful method of increasing germination for only one species in our trial, *Aquilegia formosa,* in combination with a 90-d cold-moist stratification period. We used a 1:100 smoke-water dilution, which had been identified as effective for germination response in a wide range of species (Flematti and others 2004; Jefferson and others 2014). Other studies have used stronger concentrations, reaching as high as 1:10 or 1:5 smoke-water dilution (Chou and others 2012). Individual species might respond differently to various concentrations, so it is possible we needed to use higher concentrations to benefit some of the tested species. Fire-adapted species in other ecosystems have shown a positive germination response to smoke-water, thus alternative smoke-water production techniques, concentrations, and application methods should be investigated, along with other fire-related germination cues. Byers and others (2004) found no significant differences in the germination rates of ash-treated *Gaillardia aristata* seeds as compared to controls; instead, they found that the mechanical disturbance of soil, from fire or otherwise, was responsible for the dramatic increase in population numbers of *G. aristata.* This and other prairie species may have a physiological dormancy broken by something other than the environmental-chemical cues found in burnt plant materials.

Stratification

Cold-moist stratification treatment had a significant effect on the 8 responsive species in our trial. Stratification length is an important aspect of plant propagation that should be considered when attempting to maximize efficiency. Producers' needs and timelines, however, must be addressed when making decisions about stratification length. Is a marginal increase in germination rate worth the increased production time involved? The stratification times suggested here (see Table 1) may provide guidance for producers interested in efficiently propagating these prairie species for restoration. The standard protocol guideline of 12-wk (84-d) stratification was too long for 7 out of 8 analyzed species, resulting in some unusable seeds. This finding agrees with other studies conducted on South Puget Sound prairie species (Drake and others 1998;

Russell 2011; Jones and Kaye 2015), suggesting that stratification for most herbaceous prairie species with unknown germination requirements needs to be shortened and monitored earlier to prevent seed loss.

The Native Plant Network (http://www.nativeplantnetwork.org) is a useful tool for growers of native species, and the site will continually improve as new protocols are added. Comparing our maximum germination rates and techniques to those found on the Native Plant Network and in other published or online sources, shows great variability in results, based on protocols, seed sourcing, and other factors (see Table 1). We suggest future studies include the purity and viability of the individual seedlots, as knowing the percentage of pure live seed will add clarity across seedlots and studies. Additionally, the age of the seedlots should be taken into consideration, because as seeds age they tend to be more sensitive to conditioning treatments (Basin and Baskin 1998). Testing these and other seed pre-treatments on conspecific seedlots of varying years is an area of future interest. We are only beginning to understand the complex set of conditions that optimize germination rates for each species. Continued development of clear protocols for a suite of regionally important restoration species will increase the feasibility and cost effectiveness of native species production on a commercial scale.

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