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Evaluating Basin Wildrye Seed Sources across Provisional Seed Zones, Native Forb Sowing
Depth on Species Performance and Improving the Accuracy of Collection Site
and Seed Lot Identification for Big Sagebrush

Scott L Jensen

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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ABSTRACT

Evaluating Basin Wildrye Seed Sources across Provisional Seed Zones, Native Forb Sowing Depth on Species Performance and Improving the Accuracy of Collection Site Identification for Big Sagebrush

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Doctor of Philosophy

Identifying genetically appropriate plant materials for seed based restoration relies on the principle of local adaptation where the objective is to match adaptive genetic characteristics to variation in ecological clines pertinent to plant establishment and persistence. In this study, basin wildrye (*Leymus cinereus* (Scribn. & Merr.) Á. Löve) sources from 25 wild populations and 4 commercial varieties were planted at 4 test sites. We assessed initial establishment and short term persistence. Plantings failed at 2 sites in both 2013 and 2014, with too few plants to quantify differences. At the remaining 2 sites, local sources had higher initial establishment in just 1 of 10 comparisons. Among commercial sources, the cultivars Magnar and Trailhead initially outperformed local pooled materials at Fountain Green but not at Nephi. Initial establishment under row cover was dramatically better than uncovered controls at both sites, but only persisted for 4 years after planting at the Fountain Green site.

The native forb study evaluated the effects of species, sowing depth and row cover on field emergence of 20 forbs. Overall, emergence was very low ranging between 0.2% and 1.0% for 16 of the 20 species. Four species exceeded 1% emergence. Depth effects were species, site and year dependent. The odds of emergence decreased with increasing depth for four species, increased for three species and were mixed between sites and years for the remaining species. The odds of emergence were better under row cover than for uncovered control plots. Depths evaluated were deeper than recommended for most species and likely hindered emergence for some species. Site and year had much more effect on observed emergence than depth or treatment.

Developing simple diagnostics to identify subspecies is key in the restoration of sagebrush ecosystems. We evaluated the SoilWeb app as a tool to identify sagebrush in the field. We evaluated the accuracy of the Richardson et.al. (2015) technique to classify sagebrush stands and evaluated data modeling strategies to improve classification accuracy. We found the SoilWeb app to be an accurate and informative tool to identify native-wild sagebrush populations. The Richardson et.al. (2015) seed weight criteria correctly classified just 19% of our sample populations to the correct subspecies. To improve upon this, we evaluated multifactor modeling using recursive partitioning and classification trees. Our most accurate classification tree correctly classified 80% of 2x *tridentata* sites but just 45% of *wyomingensis* sites.

Keywords: local adaptation, basin wildrye, sowing depth, big sagebrush, seed weight

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CHAPTER 1

Does Basin Wildrye Show Local Adaptation when Deployed According to Generalized Provisional Seed Zones in the Central Basin and Range Ecoregion?

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ABSTRACT

Identifying genetically appropriate plant materials for seed based restoration relies on the principle of local adaptation, where the objective is to match adaptive genetic characteristics to variation in ecological clines pertinent to plant establishment and persistence. Seed zone maps delineate some of these relationships. Generalized provisional seed zones (GPSZ) were developed for use where species-specific seed zones are lacking, as was the case for basin wildrye at the initiation of this study. In this study, basin wildrye (*Leymus cinereus* (Scribn. & Merr.) Á. Löve) sources from 25 wild populations and 4 commercial varieties were planted at 4 test sites representing the species distribution across GPSZ in the central basin and range ecoregion. Sources were seeded separately into 5 blocks for each of 2 treatments and data were grouped by tetraploid or octoploid cytotype and local or nonlocal origin for comparisons. Treatments included coverage with permeable row cover fabric or uncovered controls. We assessed initial establishment and short term persistence. Plantings failed at 2 sites in both 2013 and 2014, with too few plants to quantify differences. At the remaining 2 sites, local sources had higher initial establishment in just 1 of 10 comparisons. By year 4 row fill between local and nonlocal sources were no different. Among commercial sources, the cultivars Magnar and

Trailhead initially outperformed local pooled materials at Fountain Green but not at Nephi. This difference was no longer evident 4 years after planting. Initial establishment under row cover was dramatically better than uncovered controls but only persisted for 4 years after planting at the Fountain Green site.

INTRODUCTION

In recent years, plant materials programs and policies have emphasized the development and deployment of genetically appropriate native plant materials (Richards 1996, Shaw et al., 2008, Rogers 2004, Oldfield 2015, Wood, Doherty, & Padgett 2015). The foundation of genetic suitability is the principle of local adaptation with the intent of exploiting genetic variation to both preserve and capitalize on spatially diverse functional traits to improve restoration outcomes (Leimu et al., 2008). In application, using genetically appropriate plant materials requires a suitable pairing of restoration sites with seed sources, so that the developing plant community provides desired ecosystem services and is resilient to disturbance. (Johnson et al. 2010b, Jones 2013).

Genecological studies are a common first step in understanding morphological and phenological variation that can be used to identify adaptive genetic differences between populations (Johnson et al. 2015, Erickson, Mandel and Sorensen 2004, Campbell 1986). In genecological studies, numerous populations of the same species are planted in 1 or more common environments and many traits, that may have adaptive significance, are evaluated. Resulting data, when paired with climate records from source population locations, are used to develop seed transfer guidelines often displayed as seed zone maps. For a number of prominent Great Basin restoration grasses, genecological work is underway (St Clair 2013, Johnson 2012,

Johnson et al., 2010a, Johnson et al., 2015) and was recently completed for basin wildrye (*Leymus cinereus* (Scribn. & Merr.) Á. Löve) (Johnson et al., 2016). Yet, where genecological work is absent, many species will, of necessity be planted without species specific seed zone recommendations (Bower et al., 2014). In these cases, surrogate approaches including similar ecosystems (Johnson et al., 2010b), ecoregions (Miller et al., 2011, Omernik 1987, Hargrove 2005), plant hardiness zones (USDA 2013), and plant adaptation regions have been suggested. A few approaches have been evaluated for different species. Seed transfer models containing climate metrics best partitioned variation for bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve) (Gibson and Nelson 2017). Provisional seed zones within Level III Ecoregions (Omernick 1987) captured more variation among source populations than either PSZ or Level III Ecoregions alone, for 5 forb species. (Kramer et al 2015)

In this study, we evaluated a leading surrogate, generalized provisional seed zones (GPSZ) (Bower 2014, Kramer 2015), as a method of matching basin wildrye source populations to representative restoration sites. GPSZ delineate areas of similar winter minimum temperature and annual aridity, characteristics important to plant adaptation. When paired with ecoregions (Omernik 1987), they partition areas into climatically and ecologically distinct units and are recommended as a starting point for developing seed transfer guidelines.

Basin wildrye was selected as the test species because it is a common restoration species (Paul Krabacker, BLM Boise, ID, personal communication) in the Intermountain region yet, its genecological seed zones had not been developed. Its distribution extends from New Mexico on the south to Saskatchewan on the north then west across all states and provinces to the Pacific Coast. Within the Central Basin and Range (CBR) ecoregion, it's known distribution ranges over 1,100 meters in elevation and across 9, level 4 ecoregions (Omernik 1987). A substantial

array of climactic and ecological variation occurs throughout this species' distribution that may lead to the development of localized adaptations between spatially or ecologically divergent populations (Hereford 2009).

To test the principle of local adaptation, we hypothesized that wild basin wildrye sources planted into test sites matching their home GPSZ would demonstrate local adaptation through higher initial establishment and better short term persistence. We also hypothesized local GPSZ pooled sources would similarly outperform the cultivars 'Magnar', 'Trailhead' and 'Continental', which originate from outside the Great Basin, and Great Basin 'Tetra' which is a composite of 31 sources from multiple GPSZ's and ecoregions. In a review of 170 local adaptation studies conducted in the Great Basin, (Baughman et al. 2019) reported local sources experienced greater survival in 67% of reciprocal studies and suggested locally sourced plants likely harbor adaptations that are immediately relevant to restoration success.

From experience using fabric row cover to improve seedling establishment in nursery beds, we included row cover as a treatment option in this experiment and hypothesized basin wildrye establishment would be better under row cover. Studies report 3 to 5 °C higher soil temperatures under row cover (Harris et al. 2015) and up to 6% more moisture (Tilley et al. 2009) compared to uncovered soils. Other studies report increased survival of spring emerging seedlings (Shock et al. 2013; Stettler 2012; Tilley et al. 2009) as well as overwintering perennial plants (Harris et al. 2015). Based on these studies and prior experience, we expect row cover will create an ameliorated germination environment to provide a second suite of environmental conditions to evaluate establishment differences among sources.

MATERIALS AND METHODS

Within the Great Basin, known populations of basin wildrye ($n = 107$) were mapped to 9 GPSZ's using ArcMap 10.1 (ESRI 2012). Five zones were poorly represented; containing 1 to 3 populations within their boundaries, while 4 zones (Table 1-1) accounted for the remaining 91.6% of populations. Bower (2014) demonstrated GPSZ's account for more plant trait variation when nested within Omernik's level III ecoregions than when assessed at broader scales. Consequently, for this study, source populations were restricted to origins within the CBR ecoregion. Basin wildrye population locations were obtained from a United States Forest Service database developed over the course of several decades of plant material work and were originally located by travel throughout the area, consultation with federal and state agency personnel or university and online herbarium sources.

Basin wildrye occurs in 2 cytotypes, tetraploid ($2n=28$) and octoploid ($2n=56$) (Ogle et al. 2012). Crossing cytotypes results in sterile seed or unstable hexaploids (Young et al. 2013). Consequently, cytotypes should not be combined when assembling multi-origin seed sources for grow out or on restoration projects. As our interest, in this study, was only to evaluate establishment and short term persistence, sources were not segregated by cytotype but data were grouped by cytotype for analysis. Across the western portion of the species distribution, Culumber (2013) genetically distinguished 3 metapopulation races corresponding to the Columbia, Rocky Mountain, and Great Basin regions. Octoploid cytotypes were more abundant in the western portion of the species' range and tetraploid cytotypes were more abundant in the east. In the Great Basin, where the Great Basin race is fully encompassed by the more broadly distributed Rocky Mountain race, both cytotypes are common. In a recently-completed common garden study (Johnson and Vance-Borland 2016), genetic variation for both cytotypes was linked

to source climates. That information was used to delineate 15 geneecological seed zones for basin wildrye.

In 2013, leaf tissue was collected from CBR populations and processed through a Partec flow cytometer to determine cytotype. Leaves were harvested, placed in zip lock bags and kept on ice while in the field then transferred to a cooler (3° C) until cytotype determination, following methods described by Richardson (2012). Leaves from 2 plants per site were processed to determine each population's cytotype. Across the western portion of the species' distribution, Culumber (2013) found no cases of mixed ploidy within populations of basin wildrye.

When developing genetically appropriate stock seed supplies, leading recommendations suggest pooling seed from at least 5 populations representing in aggregate more than 50 parents (Withrow-Robinson 2006, Brown 1995, Johnson et al., 2010b). Basin wildrye populations were mapped according to seed zone and cytotype to identify populations for each zone/cytotype combination. For this study, we were not able to locate 5 populations for each GPSZ/cytotype combination (Table 1-1), nevertheless all had in excess of 100 parents.

'Magnar', 'Trailhead' and other source identified populations of basin wildrye have historically been seeded extensively in the Great Basin, especially following fire. To minimize the possibility of collecting planted sources of these materials, we excluded populations that occurred within evident fire perimeters, adjacent to roadsides, within boundaries of federal restoration projects (Pilliod et al., 2013) and visited with land owners or ranch managers about seeding history on private lands.

In 2013 and 2014, basin wildrye seed was harvested from a minimum of 50 individual plants at 25 wildland populations. Seed from each source was maintained separately, cleaned using a Clipper laboratory seed cleaner and Carter Day fractionating aspirator and viability tested (TZ) at

the Utah State Seed Lab. Commercial certified basin wildrye sources, ‘Magnar’, ‘Trailhead’ and ‘TETRA’ were acquired from the Utah Division of Wildlife Resources, Great Basin Research Center, seed warehouse.

Test Sites

Test sites were located in each of the 4 GPSZ’s with basin wildrye population occurrence rate over 5%. The Spanish Fork, Utah location represented zone 15-20 Deg. F. / 3-6 and occurred on a Timpanogos loam soil type (Farmlogs 2018a). It averaged 43 cm of annual precipitation over the last 10 years (Farmlogs 2018b). The Nephi, Utah location represented zone 15-20 Deg. F. / 6-12 and occurred on Nephi silt loam soil and averaged 28 cm of annual precipitation. The Orovada, Nevada location represented zone 20-25 Deg. F. / 6-12 and occurred on the Snapp-McConnel-Adeliade soil association and averaged 16.5 cm of annual precipitation. The Fountain Green, Utah location represented zone 10-15 Deg. F. / 6-12 and occurred on Keigley silty clay loam (Farmlogs 2018a) and averaged 33 cm of annual precipitation over the last 10 years (Farmlogs 2018b).

In May of 2013 and 2014, plots at each site were disked to incorporate existing vegetation and summer fallowed. In early fall, the sites were harrowed and roller packed to prepare the seedbed for planting. Several weeks prior to planting, plots were treated with the nonselective herbicide glyphosate to remove fall-germinated seedlings. Glyphosate was chosen for its ability to control a broad spectrum of grasses and broadleaf plants while also strongly binding to soil particles rendering it biologically unavailable with no soil residual activity (Monsanto 2004). Planting occurred the first half of November in both years. In 2014, plots were mowed in May at a 10 cm stubble height to limit weed seed maturation.

Row Cover Treatment

DeWitt's 51 g m²-1 (1.5 oz. yd²-1) N-Sulate (DeWitt Co., Inc., Sikeston, MO 63801) is a medium weight, permeable, UV-treated fabric designed to offer frost protection to plants, reduce evaporation rate, lengthen harvest time or extend flowering season. Following seeding, beds were enclosed with 3.65m wide row cover using a tractor drawn plastic mulch layer. The cover remained in place through early spring.

Study Design

A primary objective of this study was to compare establishment success among populations seeded in a manner similar to a restoration planting. This approach differs from most reciprocal studies, which are established using transplanted nursery grown stock. Transplants, particularly suitable from a research design perspective, permit evaluation of mature plant performance and longevity, but bypasses the seedling life phase. Establishing the study by direct seeding permits evaluation of this critical establishment phase, upon which all subsequent performance and longevity data rely.

The study was implemented as a 2 factor factorial that included row cover treatment and seed source in a split plot design. Ten whole plots (blocks) were assigned treatments of row cover (n=5 blocks) or uncovered controls (n=5 blocks) and seed sources were randomly assigned in subplots. In 2013 and 2014, at each site, each of 25 seed sources was planted at a density of 68.3 pure live seeds per m (20.8 pls/ft.) along a 1.83 m (6') row length. Seed was sown 2 cm deep with a tractor drawn custom Hege 1000 series cone seeder planting through John Deere® double disc openers.

Data Collection and Analysis

Year 1 establishment data, recorded as the total number of individual plants occurring in each row, were collected in the fall of each year following seeding. Short term persistence data (Year 4), measured as linear centimeters of row occupied by basin wildrye, was recorded in the fall of 2017, 4 years post 2013 planting and 3 years post 2014 planting. Sources were classified as local if they originated in the same GSPZ as the test site. For analysis, data from individual sources are either evaluated separately or pooled by cytotype or originating GPSZ to address hypotheses of interest. For example, when evaluating establishment differences at Fountain Green between local (10-15 Deg. F. / 6-12) octoploids and nonlocal (15-20 Deg. F. / 6-12) octoploids, data from 5 populations were grouped to create the local octoploid data set and data from 3 populations were grouped to create the nonlocal octoploid data set. Tetraploid and octoploid groupings were analyzed separately due to the genetic constraints of mixing these cytotypes. Data were analyzed using SAS's Mixed Procedure (SAS Institute, 2004) as a split plot with cover treatments as whole plots and sources as sub plots. Site, year, cover treatment, and source were considered fixed effects while block was considered a random effect.

Precipitation data was assembled from Farmlogs (Farmlogs 2018b) by delineating study sites and using the built in rainfall feature. Farmlogs report National Oceanic and Atmospheric Administration (NOAA) data which is calculated using radar and ground stations to algorithmically predict the amount of precipitation that falls on a high-resolution (1km) grid of the United States.

RESULTS

Year 1 Establishment

At both the Orovada and Spanish Fork sites in 2013 and 2014, spring seedling emergence was very limited and subsequent mortality was high. By fall, when Year 1 data was recorded, too few plants remained to make valid statistical inferences. For the remaining sites, we compared Year 1 establishment between pooled local GPSZ sources (occurring within the same GSPZ as the test site) and nonlocal (originating from other GPSZ) tetraploid sources, pooled local and nonlocal octoploid sources, and local tetraploid groups or local octoploid groups versus commercial sources (Tables 1-1 and 1-2). At Fountain Green, significant differences were observed between sources, treatments, years and the treatment*year interaction (Table 1-2). Among octoploid sources, local and nonlocal pooled GPSZ sources performed similarly ($p > 0.800$) (Table 1-3). The cultivars ‘Magnar’ ($p = 0.000$) and ‘Trailhead’ ($p = 0.078$) outperformed local octoploids averaging 10.2 or 5 more plants per plot respectively. Among tetraploid sources, local pooled GPSZ sources from zones 15-20 Deg. F. / 3-6 ($p = 0.054$) and 20-25 Deg. F. / 6-12 ($p = 0.121$) averaged 3.5 and 2.8 more plants per plot than local sources, respectively. ‘Magnar’ ($p = 0.000$) and ‘Trailhead’ ($p = 0.090$) outperformed local tetraploids averaging 10.1 or 4.9 more plants per plot respectively. ‘Tetra’ averaged > 3 more plants per plot but with p values > 0.26 .

Establishment in row cover treatments was better in 2014 than 2013 (Table 1-4) and establishment in row cover treatments was better both years than establishment in control plantings in 2014 and 2013, which were also different. Contrasting with row cover treatments, establishment in control was higher in 2013 than 2014 causing the significant treatment*year interaction (Tables 2 and 4).

For Year 1 establishment at Nephi, significant differences were observed between treatment, year and the interaction treatment*year (Table 1-2). Source had less of an effect, with p values typically exceeding 0.2 except where local tetraploids averaged 2.3 more plants per row than the nonlocal 10-15 Deg. F. / 3-6 (p=.060) source. Similar to the Fountain Green site, establishment in row cover treatments was better in 2014 than 2013 and establishment in row cover treatments was better both years than establishment in control plantings which did not differ by year (Table 1-4). Contrasting with row cover treatments, establishment in control was greater, though not significantly so, in 2013 than 2014 (Table 1-4) causing the significant treatment*year interaction (Table 1-2).

Short-Term Persistence

At Fountain Green there were no differences among sources in short term persistence (Year 4), measured as linear centimeters of row occupied by basin wildrye (Table 1-2). There were significant differences at Year 4 for treatment, year, and the treatment*year interaction (Table 1-2). Year 4 was higher in row cover than control plots both years (Table 1-4). Year 4 was higher in 2013 than 2014 row cover plots. Control plots were similar between years. Contrasting to row cover treatments, establishment in control was greater, though not significantly so, in 2014 than 2013 causing the significant treatment*year interaction (Table 1-4). At Nephi, there were only differences between years (Table 1-2) where Year 4 was better in 2014 than 2013 for both treatments (Table 1-4).

DISCUSSION

Unfortunately, failed plantings at Orovada and Spanish Fork reduced the scope of inference to half of what was intended. The ultimate cause of failure is unknown though observed emergence was minimal at these sites. Previous studies have shown basin wildrye is slow to germinate under field conditions, in a variety of plant community types (Cline et al. 2018) and stand establishment benefitted from supplemental irrigation (Roundy 1985) during a dry spring. Alternatively, post germination conditions may have contributed to poor establishment. Seed may have germinated during fall or winter months and perished from desiccation, frost (Roundy & Madsen 2016), or competition or allelopathic effects from weedy species (Sturm et al. 2018, Thomson et al 2017). Basin wildrye is a poor competitor during establishment (Robins et al. 2013) when growing with competitive species (Ogle et al. 2012) and at both the Orovada and Spanish Fork sites, even following efforts to reduce the seedbank, there was considerable competition from weedy species. Both locations appear suitable to support this grass with remnant native stands persisting adjacent to the Orovada test site on similar soil and topography and a planted field of basin wildrye persisting near the Spanish Fork plot on similar soils. With these sites failing to contribute data to the objective, their singular contribution is merely to provide further observation that basin wildrye is a poor competitor during establishment.

A primary objective of this study was to test whether generalized provisional seed zones geographically represent relevant partitions of selective gradients that basin wildrye has evolved under, and thereby aptly partition populations into adaptive groups. We hypothesized local adaptation expressed by pooled local sources would perform better initially and over time, than pooled nonlocal sources. Additional objectives were to compare local pooled GPSZ sources to

the commercially available sources 'Trailhead', 'Magnar', 'Continental' and Great Basin 'Tetra' and evaluate row cover as a method to improve seedling establishment.

We expected local adaptation would first be expressed by differential establishment rates with local climate benefitting local sources. But, amid 10 comparisons, local sources only outperformed nonlocal sources 1 time, among tetraploids at Nephi ($p=0.060$). To ensure results from pooled sources were not obscured by poor performing individual sources, we compared pooled local GPSZ sources to local individual sources and again found no source differences. In other words, local individual sources, performed similar to local pooled sources at these 2 sites. At Fountain Green, the commercial sources 'Magnar' and 'Trailhead' initially established better than local sources but those differences were no longer evident by Year 4.

The intent of seed zones is to match seed sources to geographic areas where they are well adapted. Over the brief duration of this study, the lack of differences suggests basin wildrye functioned as a habitat generalist, unresponsive to seed zones. Selective gradients, if present or expressed during the study interval, did not exert persistent differential results. It is possible that documenting such may require more annual replication and longer persistence intervals to capture climatic variability sufficient to cause selective pressure. For Basin wildrye, empirical seed zones are now available (Johnson and Vance-Borland 2016) and more refined field testing is needed to determine the significance of seed zones for this species.

Row cover treatments dramatically improved Year 1 establishment over control treatments both years and at both sites. The most modest improvement was 318%, at Nephi in 2013 (Table 1-4). This demonstrates the row cover treatment met our objective of creating a second suite of germination environments, providing additional opportunity to evaluate seed source performance. Yet given multiple years, sites and germination environments, our local advantage

hypothesis did not bear out. Row cover is not practical at landscape scales commonly associated with revegetation projects but could be utilized on small projects or at selected locations within larger projects to improve initial establishment.

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TABLES

Table 1-1. Each test site and the generalized provisional seed zone it resided in is listed. The number (n) of basin wildrye populations of each cytotype originating from each provisional seed zone is shown.

Site	Local Source	n	Cytotype
Fountain Green	10-15.Deg.F./6-12	4	Tetraploid
		5	Octoploid
Nephi	15-20.Deg.F./6-12	3	Tetraploid
		3	Octoploid
Spanish Fork	15-20.Deg.F./3-6	5	Tetraploid
		0	Octoploid
Orovada	20-25.Deg.F./6-12	3	Tetraploid
		2	Octoploid

Table 1-2. F and P values for testing overall effects of Year 1 establishment and Year 4 persistence for basin wildrye populations. Year 1 establishment data was recorded as the total number of individual plants occurring in each row, collected in the fall of 2014 and 2015 approximately 10 months after seeding. Year 4 persistence was measured as linear centimeters of row occupied by basin wildrye. It was recorded in the fall of 2017, 4 years post 2013 planting and 3 years post 2014 planting. Source effects compare local basin wildrye seed sources to nonlocal sources and local sources to cultivars. For example, when evaluating establishment differences at Fountain Green between local (10-15 Deg. F. / 6-12) octoploids and nonlocal (15-20 Deg. F. / 6-12) octoploids, data from 5 populations (Table 1-1) were grouped to create the local octoploid data set and data from 3 populations were grouped to create the nonlocal octoploid data set. Tetraploid and octoploid groupings were analyzed separately due to the genetic constraints of mixing these cytotypes. Treatments were plots covered with row cover or uncovered control plots.

Site	Effect	Year 1 Establishment		Year 4 Persistence	
		F Statistic	P Value	F Statistic	P Value
Fountain Green					
	Source	1.76	0.0104	0.918	0.5885
	Treatment	372.86	<.0001	56.871	<.0001
	Source*Treatment	1.21	0.2103	4.011	0.9798
	Year	11.08	0.0009	0.525	0.0458
	Source*Year	1.15	0.273	0.48	0.9898
	Treatment*Year	26.14	<.0001	5.867	0.0158
	Source*Treatment*Year	0.98	0.498	0.408	0.9973
Nephi					
	Source	1.19	0.2376	0.283	1
	Treatment	422.12	<.0001	2.332	0.127
	Source*Treatment	1.16	0.2665	92.717	0.999
	Year	227.62	<.0001	0.354	<.0001
	Source*Year	0.7	0.872	0.247	1
	Treatment*Year	238.17	<.0001	0.004	0.948
	Source*Treatment*Year	0.76	0.8106	0.242	1

Table 1-3. T and P values for testing source effects on Year 1 establishment of basin wildrye populations. The estimate is the difference in the average number of plants per row as described by source comparisons. Positive values indicate a higher mean establishment value for the local source and negative values indicate higher mean values for the compared source. Year 1 establishment data was recorded as the total number of individual plants occurring in each row. Sources were classified as local if they originated in the same GSPZ as the test site. For analysis, data from individual sources are either evaluated separately or pooled by cytotype or originating GPSZ to address hypotheses of interest. For example, when evaluating establishment differences at Fountain Green between local (10-15 Deg. F. / 6-12) octoploids and nonlocal (15-20 Deg. F. / 6-12) octoploids, data from 5 populations were grouped to create the local octoploid data set and data from 3 populations were grouped to create the nonlocal octoploid data set. Tetraploid and octoploid groupings were analyzed separately due to the genetic constraints of mixing these cytotypes.

Site	Cytotype	Source comparisons	Estimate	DF	t Value	Pr> t
Fountain Green (10-15 Deg. F. /6-12)			plants/ row			
	Octoploids	Local vs. 15-20 Deg. F. / 6-12	0.0330	460	0.02	0.9859
		Local vs. 20-25 Deg. F. / 6-12	0.5250	460	0.24	0.808
		No sources from 15-20 Deg. F. / 3-6				
		Local vs. 'Continental'	0.2500	460	0.09	0.9296
		Local vs. 'Magnar'	-10.1500	460	-3.56	0.0004
		Local vs. 'TETRA'	-3.1500	460	-1.11	0.2657
		Local vs. 'Trailhead'	-5.0000	460	-1.77	0.0776
	Tetraploids	Local vs. 15-20 Deg. F. / 3-6	-3.5250	460	-1.93	0.054
		Local vs. 20-25 Deg. F. / 6-12	-2.8375	460	-1.55	0.1206
		Local vs. 15-20 Deg. F. / 6-12	1.1333	460	0.58	0.5656
		Local vs. 'Continental'	0.3500	460	0.12	0.9035
		Local vs. 'Magnar'	-10.0500	460	-3.48	0.0005
		Local vs. 'TETRA'	-3.0500	460	-1.06	0.291
		Local vs. 'Trailhead'	-4.9000	460	-1.7	0.0901
Nephi (15-20 Deg. F. /6-12)						
	Octoploids	Local vs. 10-15 Deg. F. / 6-12	-1.3767	460	-1.06	0.2881
		Local vs. 20-25 Deg. F. / 6-12	-0.7417	460	-0.46	0.6469
		No sources for 15-20 Deg. F. / 3-6				
		Local vs. 'Continental'	-2.5167	460	-1.23	0.2194

		Local vs. 'Magnar'	-0.9667	460	-0.47	0.6369
		Local vs. 'TETRA'	-1.8167	460	-0.89	0.3752
		Local vs. 'Trailhead'	-2.5167	460	-1.23	0.2194
	Tetraploids	Local vs. 10-15 Deg. F. / 3-6	2.3625	460	1.89	0.0601
		Local vs. 15-20 Deg. F. / 3-6	-0.3000	460	-0.024	0.8109
		Local vs. 20-25 Deg. F. / 6-12	1.0958	460	0.81	0.4186
		Local vs. 'Continental'	-0.0375	460	-0.02	0.9849
		Local vs. 'Magnar'	1.5125	460	0.76	0.4457
		Local vs. 'TETRA'	0.6625	460	0.33	0.7383
		Local vs. 'Trailhead'	-0.0375	460	-0.02	0.9849

Table 1-4. T and P values for testing Treatment*Year effects on Year 1 establishment and Year 4 persistence of basin wildrye populations. Year 1 establishment data, recorded as the total number of individual plants occurring in each row were collected in the fall of each year following seeding. Year 4 persistence data measured as linear centimeters of row occupied by basin wildrye, was recorded in the fall of 2017, 4 years post 2013 planting and 3 years post 2014 planting.

Site	Treatment*Year Effect		Estimate	Pr> t
Fountain Green				
Year 1			Plants / row	
	Row Cover	2013	17.7034 B*	<.0001
	Row Cover	2014	25.7931 A	<.0001
	Control	2013	4.0966 C	0.0149
	Control	2014	2.3862 D	0.1551
Year 4			cm plant / row	
	Row Cover	2013	91.1862 A	<.0001
	Row Cover	2014	69.8689 B	0.0012
	Control	2013	43.1862 C	0.3692
	Control	2014	45.2068 C	0.7385
Nephi				
Year 1			Plants / row	
	Row Cover	2013	4.5931 B	<.0001
	Row Cover	2014	24.6828 A	<.0001
	Control	2013	1.2276 C	0.1058
	Control	2014	1.0000 C	0.1875
Year 4			cm plant / row	
	Row Cover	2013	20.1793 B	0.0138
	Row Cover	2014	61.1862 A	<.0001
	Control	2013	13.3517 B	0.0277
	Control	2014	54.9172 A	<.0001
* Letters indicate significant differences within sites.				

CHAPTER 2

Emergence of 20 Great Basin Native Forbs When Sown at Increasing Depths

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ABSTRACT

This study, conducted at three sites in 2013 and 2014, evaluated the effects of species, sowing depth and row cover on field emergence of 20 forbs native to the Great Basin. Half of the plots were enclosed with row cover fabric and half were left uncovered. The largest number of seedlings counted the spring or fall following the planting year was the response variable. We hypothesized that increasing seeding depth would result in lower emergence and that emergence would be better under the row cover treatment. Overall, emergence was very low ranging between 0.2% and 1.0% of seed sown for 16 of the 20 species. Four species exceeded 1% emergence. Depth effects were species, site and year dependent. The odds of emergence decreased with increasing depth for four species, increased for three species and were mixed between sites and years for the remaining species. The odds of emergence were better under row cover than for uncovered control plots, but varied by species, site and year. Depths evaluated were deeper than recommended for most species and likely hindered emergence for some species. Site and year had much more effect on observed emergence than depth or treatment.

INTRODUCTION

Federal policy shifts (Plant Conservation Alliance 2015) encouraging the use of native plant materials have resulted in a substantial increase in demand for native seed. While workhorse restoration species remain primarily graminoids (Galavan and Roller 2016), there is growing interest in diversifying seed mixes with native forbs to better meet multiple use objectives (Olwell and Bosak 2015). Forbs contribute to resilient, biologically diverse plant communities that are both enjoyed for their beauty and required as components of critical habitat (Connelly et al. 2000). Consequently, native forbs have become common species in western federal plant materials programs, led by the Bureau of Land Management (CPNPP 2017) and USDA Forest Service (GBNNP 2017), where testing and evaluation are being conducted to understand germination cues, establishment requirements, agronomic potential, and species suitability in restoration contexts (GBNNP 2017). Species selected for this study (Table 2-1) either have some history of use in restoration plantings like yarrow (*Achillea millefolium* L.), globemallows (*Sphaeralcea grossulariifolia* (Hook. & Arn.) Rydb. and *Sphaeralcea munroana* (Douglas) Spach), and lupines (*Lupinus argenteus* Pursh) or are being screened by the Great Basin Native Plant Program (GBNPP) as candidate species for further development.

In rangeland settings only a small percentage of sown seed establishes as plants. Among common restoration species, wheatgrasses express good establishment abilities yet seedling establishment is typically less than 10% (Cook et al.1967). Among several shrubs, seedling establishment for plantings in southern Wyoming ranged between 0.01 to 3.30% (Luke and Monsen 1984). In central Nevada fourwing saltbush established at 10% while big sagebrush and rubber rabbitbrush established at less than 3.5% (Monsen and Richardson 1984). Among factors affecting seedling establishment are high annual and season variations in precipitation and

temperature (Hardegree et al. 2016, Roundy & Madsen 2016), competition from weedy species and existing vegetation (Davies and Bates 2014) and rodents (Gurney et al. 2015).

Seeding depth recommendations available from technical references (Stevenson 2012, Monsen et al. 2004, Jensen et al. 2001, Ogle et al. 2008a, 2008b) are based on rules of thumb relating appropriate seed placement to seed size. Generally, shallow seeding depths are recommended for small seeds and deeper depths are recommended with increasing seed size. III effects of seed placement can occur if seed is planted either too shallow or too deep. The art of planting is locating species-specific depths where germination and growth occur more rapidly than competing causes of mortality and plant vigor is not hampered by poor root development. A review of studies comparing aerial seeding to drill seeding reported drill seeding outperformed broadcast seeding 73% of the time (Hardegree et al., 2011) although these studies primarily represent seeded grasses. The lack of adequate seed to soil contact and the volatile nature of soil moisture and temperature typically hinder germination of surface sown seed. Countering this, deep planting may decrease emergence (Redmann & Qi, 1992, Berti & Johnson, 2013) or inhibit emergence altogether as deeper planted seed requires increased energy to reach the soil surface where photosynthesis can begin. Reported depth effects on restoration species are largely restricted to graminoids (Hull 1964, Limbach & Call 1995) and often from greenhouse studies rather than field settings. Results from greenhouse and field studies can be quite disparate (Berti & Johnson, 2013) due in part to differences in the variability of moisture, temperature and soil microbes that are more easily monitored and controlled in a greenhouse environment. Weed science offers the most thorough treatment of seeding depth effects on dicots, showing specific results for many species (Grundy et al. 2003; Boyd & Van Acker, 2003) but information on native forbs is rare. Basalt milkvetch (*Astragalus filipes* Torr. ex A. Gray) and gooseberryleaf

globemallow were the only species included in this study with depth effects reported in the literature. In greenhouse studies, seedling emergence of basalt milkvetch was lower when planted at 19 mm than at 6 mm (Bushman et al., 2015) while gooseberryleaf globemallow emerged better when sown at depths between 6 and 12 mm than surface sown in a sandy loam soil (Rawlins et al. 2009).

Soil texture has been shown to affect emergence with detrimental effects typically associated with higher clay contents. For Jimsonweed (*Datura stramonium* L.), germination inhibition was directly proportional to clay content and inversely proportional to sand content as seeding depth increased (Benvenuti, 2003). Similarly two prairie clovers (*Dalea ornata* Douglas ex Hook. and *Dalea searlsiae* A. Gray) emerged better in sandy soils than soils with higher clay content (Bushman et.al 2015).

Soil temperature and water potential have been used to model germination response of rangeland species (Hardegree et al. 2018, Cline et al. 2018, Rawlins et al. 2012b, Rawlins et al. 2012a). These models generally show germination requirements are met under a variety of rangeland plant community types, yet mortality inducing events, like routine frosts (Roundy & Madsen 2016) hinder establishment. Novel treatments are being applied to seed to delay germination (Richardson et al. 2019) until the risk of frost diminishes. In this study we incorporate DeWitt's (51 g m⁻²-1) N-Sulate (DeWitt Co., Inc., Sikeston, MO 63801) fabric designed to offer frost protection and reduce evaporation. The material is a medium weight, permeable, UV-treated fabric also used in the horticultural industry to lengthen harvest time or extend flowering season. In the Intermountain area, the USFS Lucky Peak nursery pioneered the use of N-Sulate as a seedbed row cover (Schmal et al. 2007). Following fall planting they roll row cover over beds using a plastic mulch layer. Studies report 3 to 5 °C higher soil temperatures (Harris et al.

2015) and up to 6% more moisture (Tilley et al. 2009) compared to uncovered soils. Other studies report increased establishment of spring-emerging seedlings (Shock et al. 2013; Stettler and Whittaker 2012; Tilley et al. 2009) and overwintering perennial plants (Harris et al. 2015).

Our objectives were to evaluate the effects of sowing depth and the treatment effect of row cover on field emergence of 20 forbs. We hypothesized that all species would perform better under row cover and that increasing depths would hinder establishment for all species. Typical rangeland establishment data has not been documented for these species. This study reports field trial emergence data, providing realistic expectations to restoration practitioners.

MATERIALS AND METHODS

Site characteristics for the Wells and Orovada, NV and Fountain Green, UT sites are reported in Table 2-2. While soil surveys (Farmlogs 2018a) report site level soil classifications as described, surface soils (0-10 cm) within seeded plots, at all sites, tested as loam using the Bouyoucos hydrometer mechanical analysis method (Bouyoucos 1962).

We evaluated 20 forbs sown at four planting depths and either uncovered or with row cover. Seeds were sown in fall 2013 and 2014. The design included treatment, seeding depth, and species in a hierarchical set of experimental units, resulting in a split-split-plot design. Whole plots consisted of a single planter pass 20 segments long and four rows wide. Five whole plots at each site had covered rows and five were left uncovered. Sub plots consisted of rows with different randomly-assigned sowing depths within whole plots. Sub-sub-plots were the 20 segment locations within each row, with the 20 species assigned at random to the segment locations.

The experiment was sown using a tractor drawn Hege seeder outfitted with four independent seeding cones and corresponding double disc openers. A full revolution of the cone deposited seed into furrows 1.5 m long (segment) and 63.5 cm apart. Each disc opener was individually adjusted to sow at one of four mechanically fixed depths; 1.4 cm, 2.6 cm, 3.6 cm, or 4.0 cm. The nonsymmetrical depth increase was due to hole spacing and not further adjustable. In May of each year, plots at each site were disked to incorporate existing vegetation and summer fallowed. In early fall, the sites were harrowed and roller packed to prepare the seedbed for planting. This created a uniform seedbed permitting the precision planting depths described above.

In prior years, late fall surface seedings at these sites were compromised by seed predators which can have dramatic impacts on restoration plantings (Hardegee et al. 2011, Monsen et al. 2004). To limit predation effects in this study, surface sown treatments were intentionally avoided. The 1.4 cm depth was the shallowest depth that provided complete soil coverage of the larger seeded species. Row cover was installed immediately following planting and remained in place until mid-April of the following spring.

Seed (Table 2-1) was acquired from USDA Rocky Mountain Research Station offices in Boise, Idaho and Provo, Utah and originated from populations within the Great Basin. Individual seed lots were tetrazolium chloride tested (TZ) for viability at the Utah State Seed Lab. These results were used to adjust individual species seeding densities to 250 pure live seeds (PLS) per 1.5-m segment. For brevity, species codes (USDA 2019) are used throughout rather than common names. A list of scientific names along with their corresponding species codes can be found in Table 2-1.

Seedling emergence counts were made during late spring and fall the year following planting. The largest number of seedlings recorded in each row across count dates was used for analysis.

Because the extremely low emergence on some sites resulted in a non-normal distribution for our response variables, we used generalized linear mixed models (GLMM) (*lme4* package (Douglas Bates 2015) (R Core team, 2018) instead of either linear mixed models or generalized linear models to model the number of emerged seedlings. Linear mixed models permit inclusion of random effects and generalized linear models permit non-normal data such as emergence counts. For analyzing non-normal data where random effects are present, generalized linear mixed models are the best tool (Bolker et al. 2009). Our model assumes that the number of emerged seedlings follows a binomial distribution with the probability of emergence (p) dependent on the values of the predictors (Species, Depth, Site, Year and Treatment) and the random effects (Block and Row). Specifically, $\text{logit}(p)$ follows a normal distribution with the predictors acting as fixed effects and Block and Row affecting the error structure.

The global model contained the following significant terms: Treatment + Species + Treatment*Species + Site + Site*Treatment + Site*Species + Year + Year*Treatment + Year*Site + Depth + Depth*Species + Block + Row. Subsequently, because the Site*Year combinations were dramatically different, individual Site*Year models were developed removing responses where seedling emergence was near zero. This approach focusses on fitting the most complete model possible based on relevant parameters to each Site*Year combination. In GLMM one value from each predictor (Species, Depth, Treatment, Site and Year) is selected as the standard against which other values are compared. We refer to this as the baseline value. For all but one model the baseline value for Species was LONU2, the baseline value for Depth was 1.4 cm, and the baseline value for Treatment was row cover. For Fountain Green in 2014 ASFI replaces LONU2 as the baseline species in the model. For the global model the baseline value for Year was 2013 and the baseline value for Site was Fountain Green. Thus the global

model intercept represents the log odds of emergence for LONU2 under row cover at Fountain Green in 2013. Model based estimates for specific groups are obtained from parameter estimates expressed as log odds. Converting a log odds parameter estimate into a meaningful metric is accomplished through an exponent transformation. For example, the global coefficient for Depth is -0.17374, so the odds of emergence is 84% as large for LONU2 ($\exp(-0.17374)=0.84$) when depth is increased by 1 cm. The actual effect of Depth changes for each species. For example, increasing depth by 1 cm for BAHO can be found by using both the Depth coefficient and the coefficient for the BAHO:Depth interaction: $\exp(-0.17374 + 0.33505)=1.175$. That is, increasing Depth by one increment (~1cm) for BAHO increases the odds of emergence by 17.5%. To facilitate easier interpretation of model output in log odds, data in Tables 3 and 6 are reported as percentage basis of the odds of emergence $(\exp(\text{coefficient})-1)*100$ where positive values indicate higher emergence values and negative values indicate lower emergence values.

Precipitation data were assembled from Farmlogs (Farmlogs 2018b) by delineating study sites and using the built in rainfall feature. Farmlogs reports National Oceanic and Atmospheric Administration (NOAA) data. Growing Degree Days (GDD) were gathered from FarmLogs (Farmlogs 2018c) data using the Heat Units tool. Farmlogs computes corn GDD by taking the average daily temperature (bounded by a minimum of 10° C and a maximum of 30° C) subtracted by 10° C to compute the average number of degrees above 10° C that the crop accumulates per day.

RESULTS

Average study wide emergence for the 20 species was low with only five species reaching 1%. Top performers were (Table 2-3) LONU2 (6.9%), LUPR2 (2.4%), LUAR3 (2.1%) PESP

(1.4%) and BAHO (1.0%). These same five species had adequate nonzero data to be included in four or more Site*Year depth analyses (Table 2-4). LONU2 was the only species with adequate nonzero data to be included in all six depth analyses. The remaining 15 species occurred in three or fewer comparisons. Emergence for four species, ENNU, LONU2, PEPA6, and STPII was penalized by increasing depth while BAHO, PEAC and SPMU saw better emergence at increasing depth. HEMUN and MACA were not observed in adequate quantities to quantify. The remaining 11 species saw either nonsignificant or both positive and negative depth relationships at different Site*Year combinations. Overall emergence was dramatically better ($P < 0.001$) in 2013 than 2014. Overall odds of emergence in 2014 were just 7.8%, 29.3% and 4.9% the odds of emergence in 2013 at Orovada, Wells and Fountain Green respectively. The odds of emergence in control plots were 44% as good as under row cover in 2013 and 21% in 2014. At Orovada, Wells and Fountain Green the odds of emergence were 2.9%, 59% and 3.4% in control plots compared to row cover respectively.

Site X Year Analyses

Orovada 2013 –Data from eight species exclusive to row cover plots were included in the Orovada 2013 model. The model terms were Species, Depth and the Species*Depth interaction all of which were significant ($P < 0.001$) (Table 2-5). Measurable emergence occurred only under row cover treatments. Increasing seeding depth improved probability of emergence for BAHO, LUAR3, LUPR2 and PESP and reduced probability of emergence for AGGR and LONU2 (Table 2-4). LONU2 had significantly higher emergence than other species, with one caveat: the model-predicted difference between AGGR and LONU2 is not significant when Depth = 1.4 cm.

Wells 2013 – Six species were removed due to near zero emergence. All factors in the model, Species, Species*Depth, Treatment *Species, Treatment, Depth, and Treatment*Depth were significant ($P < 0.05$). Row cover significantly increased the probability of emergence. For LONU2, the odds of emergence in control plots was 38% the odds of emergence in row cover plots. The penalty for not being covered varied by species but was always detrimental (Table 2-6). LONU2 had the highest emergence regardless of treatment. Depth effect varied by species, with three species experiencing lower odds of emergence with increasing depth and seven species showing improved emergence odds with increasing depth. The change in depth effect across treatments was significant ($p = 0.0187$) but relatively small. The impact of increasing depth was slightly more negative for control (-64.6%) than it was for row cover plots (-61.8%).

Fountain Green 2013 – Nine species were dropped where emergence was too low. The model factors Species, Treatment*Species, Species*Depth, Depth are significant ($P < 0.001$). Row cover outperformed control plots for all species, with the control penalty exceeding 90% for each species. SPGR was the highest emerging species under row cover and a significantly better performer than LONU2. LONU2 had highest emergence in the control group. Depth had a significant detrimental effect on LONU2 under row cover, with a one unit increase in depth reducing odds of emergence by 11%. ENNU, LONU2, PEPA6, SPGR and STPII showed lower emergence from increasing depth while LUPR2 and SPMU had higher emergence with increasing depth. The effects of increasing depth under control varied across species, but generally, the impact was less detrimental or even positive for some species.

Orovada 2014 - Nine species and the entire control group were removed due to near zero emergence. Species and Species*Depth were significant factors ($P < 0.001$). Depth was only significant as an interaction term. LONU2 had significantly higher emergence than all other

species. Increasing planting depth had a positive impact on four species, ERUM, LUAR3, SPGR, and SPMU, with a one unit increase in depth resulting in odds of emergence increased by 118%, 52%, 41% and 124% respectively.

Wells 2014 – This analysis was restricted to ASFI, BAHO, LONU2, LUAR3, and LUPR2 in just the row cover group. Species, Depth and Depth*Species were significant ($P<0.05$). LONU2 had significantly higher emergence than other species. A one unit increase in depth reduced the odds of emergence by 31% for LONU2 and increased the odds of emergence by 53% for BAHO. The remaining species were not significantly affected by increasing depth.

Fountain Green 2014 – This analysis used LONU2, LUAR3, SPGR and SPMU in both row cover and control groups. ASFI replaces LONU2 as the baseline species in this model. Species, Species*Depth, and Treatment*Species terms are significant ($P<0.05$). Depth and treatment are only important in that their interactions with species are significant. ASFI had significantly higher emergence than the other three species. Increasing seeding depth had a negative effect on ASFI and a positive effect on SPGR and SPMU, with a one unit increase in depth reducing emergence odds by 163% or increasing emergence odds by 110% or 87% respectively. All species performed better under row cover except LUAR3 which emerged equally well in control plots.

DISCUSSION

The global model indicates the contribution of each model term to seedling emergence. In GLMM comparing effect sizes among terms can be done by evaluating size differences between mean squares. For our global model, the largest mean square values are for Year (3823.4) and Site (1596.3). These effects appear to be driven by annual and local weather patterns. Overall

odds of emergence in 2014 were just 7.8%, 29.3% and 4.9% ($p < 0.001$) the odds of emergence in 2013 at Orovada, Wells and Fountain Green, respectively. In 2014, during the seven week period beginning March 1st – April 21st, Wells and Orovada received less than 20 mm (19.3 and 18.8 mm respectively) of precipitation and Fountain Green only 6.6 mm (Farmlogs 2018b) compared to average or above normal precipitation in 2013. Rainfall was consistent and actually more abundant the last week of April through May in 2014 than in 2013, but moisture that late in the spring was apparently too late to benefit emergence of the study species.

The volume and timing of precipitation at Wells along with cooler temperatures likely extended the duration of seedbed moisture availability contributing to better emergence. Spring precipitation (March - May) was greater at Wells in both 2013 (92.7 mm) and 2014 (123.7 mm) compared to Fountain Green (55.4 mm, 86.1 mm) and Orovada (81.8 mm, 90.7 mm) (Farmlogs 2018b). In this and other work, across a range of sites, study species have been observed to germinate from March 1st – April 21st. During this critical interval, NOAA data from 2013 indicated that Wells received three rainfall events > 2.5 mm totaling 35.6 mm (Farmlogs 2018b). During the same interval Fountain Green received two rainfall events > 2.5 mm totaling 24.9 mm, and Orovada had four events > 2.5 mm totaling 57.2 mm. While the Orovada site received more rainfall, temperatures were also warmer. Orovada had accumulated 243 GGD's by April 21st while Fountain Green accumulated 170 and Wells accumulated just 163 (Farmlogs 2018c). Warmer temperatures and sandier soils at Orovada likely resulted in more rapid drying of the seedbed than at other sites.

Across species, row cover improved emergence odds, though not always significantly (Table 2-6). The odds of emergence in control plots were 44% as good as under row cover in 2013 and

21% in 2014. At Orovada, Wells and Fountain Green the odds of emergence were 2.9%, 59% and 3.4% in control plots compared to row cover respectively.

In descending order of magnitude, the mean squares for Year, Site, Species and Treatment (Table 2-5) are about 890, 370, 150, and 62 times larger than the mean squares for Depth. Interacting with other terms, Depth was of moderate value in explaining emergence variation. Of particular interest are the varied Depth*Species interactions. ENNU, LONU2, PEPA6, and STPII emerged better at 1.4 cm than deeper depths. AGGR, ASFI, HEMUN, and MACA may fall into this group as well, although they showed mixed results. BAHO, PEAC and SPMU emerge better at deeper depths. ERUM, LUAR3, LUPR2, PEAC and SPGR may group with these species but also saw mixed results.

The change in depth effect across treatment was significant in two cases, Fountain Green ($P < 0.001$) and Wells ($P = 0.014$) 2013 but the effects were opposed. In the first case increasing depth under control was less detrimental or positive for some species while in the latter was slightly more detrimental.

This study is unique in that it provides data on seedling emergence for forb species of restoration interest in the Intermountain West under field conditions, thereby providing realistic approximations to restoration outcomes. This information is largely unavailable in literature even for commonly used restoration species yet of marked utility to restoration practitioners. While this study was well replicated it is temporally limited to two years, spatially limited to three sites, focused on loam soil textures and excluded surface planted treatments. The most prominent differences in emergence were first due to year then to site, and in both cases spring precipitation patterns appear to be the driving influence. Of factors that are controllable, both the use of row cover and pairing appropriate planting depth to individual species preference were beneficial.

Row cover could be used to improve establishment of diversity islands but is of little practical use on rangeland seedings at typical scales. A handful of species, namely barestem biscuitroot, hairy bigleaf lupine, silvery lupine, royal beardtongue and Hooker's balsamroot saw average emergence above 1%. For restoration practitioners accounting for the additional factors of seeds/kg and price offers more relevant information than emergence alone. Table 2-7 estimates costs for 100 plants computed from emergence data, seed weights and price estimates for a subset of species and highlights cost differences among species. Silvery lupine, one of the better emerging species is among the most expensive, whereas some of the low emerging species, like globemallows, cost less after accounting for seed price and seed weight.

In this study mean emergence rates for all species were low with most species not achieving 1% meaning, for most species, nearly 99% of seed failed to transition to visually-detected-emerged seedlings. This is typical of rangeland plantings. Low transition rates were reported for commonly seeded grasses (James et al. 2011). Enormous potential exists in understanding species specific germination and establishment requirements and capitalizing on these essentials to transition more seed to plants. Research efforts focused on this life stage are likely to yield the most benefit to improved restoration success.

IMPLICATIONS FOR PRACTICE

- Sowing seed at appropriate depth benefitted seedling emergence for several native forbs.
- *Enceliopsis nudicaulis*, *Lomatium nudicaule*, *Penstemon pachyphyllus*, and *Stanleya pinnata* var. *integrifolia* had lower emergence as depth increased beyond 1.4 cm.
- *Balsamorhiza hookeri*, *Penstemon acuminatus*, and *Sphaeralcea munroana* experienced higher emergence as depth increased from 1.4 to 4 cm.

- Row cover fabric had neutral to positive benefits on seedling emergence. When positive the effects were generally quite dramatic.
- For restoration practitioners accounting for the additional factors of seed weight and price offers more relevant information than emergence alone.

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National Plant Data Team, Greensboro, NC 27401-4901 USA.

TABLES

Table 2-1. Scientific names of study species are listed along with species code and recommended sowing depths obtained from literature.

Scientific Name	Species code	Recommended sowing depth (cm)
<i>Achillea millefolium</i>	ACMI	≤ 0.64
<i>Agoseris grandiflora</i>	AGGR	no information
<i>Agoseris heterophylla</i>	AGHE2	no information
<i>Arenaria macradenia ssp. ferrisiae</i>	ARMAF	0.64 - 1.27
<i>Astragalus filipes</i>	ASF1	0.64
<i>Balsamorhiza hookeri</i>	BAHO	1.27
<i>Enceliopsis nudicaulis</i>	ENNU	no information
<i>Eriogonum umbellatum</i>	ERUM	0.64
<i>Heliomeris multiflora var. nevadensis</i>	HEMUN	0.64
<i>Ipomopsis aggregata</i>	IPAG	0.64 - 1.27
<i>Lomatium nudicaule</i>	LONU2	no information
<i>Lupinus argenteus</i>	LUAR3	1.27 - 1.90
<i>Lupinus prunophilus</i>	LUPR2	1.27 - 1.90
<i>Machaeranthera canescens</i>	MACA	< 0.64
<i>Penstemon acuminatus</i>	PEAC	0.32 - 0.64
<i>Penstemon pachyphyllus</i>	PEPA6	0.32
<i>Penstemon speciosus</i>	PESP	0.32 - 0.64
<i>Sphaeralcea grossulariifolia</i>	SPGR	0.64 - 1.27
<i>Sphaeralcea munroana</i>	SPMU	0.64 - 1.27
<i>Stanleya pinnata var. integrifolia</i>	STPII	0.64 - 1.27

Table 2-2. Study site characteristics of elevation, precipitation and soil type and texture are reported.

Study Site Location	Elevation	Precipitation	Soil Series	Soil Texture
Wells, Nevada	1816 m	305 mm	Hunnton–Wieland	silt loam, loam
Orovada, Nevada	1457 m	320 mm	Snapp-McConnel-Adelaide	very fine sandy loam
				fine sandy loam and silt loam
Fountain Green, Utah	1746 m	330 mm	Keigley	silty clay loam

Table 2-3. Mean emergence (%) of forbs sown for different sites, years, and in row cover or control plots. Empty cells represent an inadequate emergence response to quantify. There were no mean emergence values in coltrol plots with *.

Species	Study Average Emergence (%)	Fountain Green				Orovada		Wells		
		2013		2014		2013*	2014*	2013		2014*
		Row Cover	Control	Row Cover	Control	Row Cover	Row Cover	Row Cover	Control	Row Cover
		Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
ACMI	0.25						0.7 \pm 0.75			
AGGR	0.59					3.85 \pm 0.36	0.85 \pm 0.82	4.9 \pm 0.24	4.05 \pm 0.16	
AGHE2	0.36	2.9 \pm .034	2.7 \pm 0.22					3.8 \pm 0.39	0.65 \pm 0.31	
ARMAF	0.29	2.15 \pm 0.40	2 \pm 0.25					4.4 \pm .033	0.25 \pm 0.46	
ASFI	0.64			3.55 \pm 0.56	0.5 \pm 0.99			8.4 \pm .024	2.25 \pm 0.18	0.25 \pm 1.22
BAHO	0.95					16.35 \pm 0.22	2.1 \pm 0.55	5.35 \pm 0.28	2.25 \pm 0.19	1 \pm 1.03
ENNU	0.73	5.55 \pm 0.26	3.55 \pm 0.19			1.25 \pm 0.65		7.95 \pm 0.22	3.2 \pm 0.16	
ERUM	0.61	11 \pm 0.25	1.65 \pm 0.22				2.25 \pm 0.78	1.95 \pm 0.73	1.4 \pm 0.26	
HEMUN	0.2									
IPAG	0.91						1.8 \pm 0.59	17.8 \pm 0.20	1.25 \pm 0.21	
LONU2	6.92	12.55 \pm 0.93	9.3 \pm 1.31			35.65 \pm .033	7.55 \pm 0.51	92.05 \pm 0.29	42.15 \pm 0.27	7.8 \pm 1.11
LUAR3	2.09			0.55 \pm 0.90	0.05 \pm 1.08	6.05 \pm 0.47	2.4 \pm 0.53	35.85 \pm 0.14	11.6 \pm 0.09	3.6 \pm 0.37
LUPR2	2.39	2.7 \pm 0.68	1.3 \pm 0.27			7.25 \pm 0.44	1.95 \pm 0.48	40 \pm 0.13	14.15 \pm 0.09	4 \pm 0.39
MACA	0.34									
PEAC	0.73							14.65 \pm 0.20	1.5 \pm 0.20	
PEPA6	0.37	2.7 \pm 0.30	2.5 \pm 0.23			2.65 \pm 0.41		2.5 \pm 0.37	0.4 \pm 0.39	
PESP	1.44	4.75 \pm 0.28	5.05 \pm 0.18			2.25 \pm .56	4.4 \pm 0.40	21.7 \pm 0.16	4.7 \pm 0.13	
SPGR	0.68	13.7 \pm 0.23	1.7 \pm 0.21	1.75 \pm 0.76	1.55 \pm 0.45		1 \pm 0.75			

SPMU	0.4	7.7 ± 0.29	1.55 ± 0.23	1.05 ± 0.91	0.6 ± 0.52		0.75 ± 1.30			
STPII	0.31	2.65 ± 0.33	1.15 ± 0.28							

Table 2-4. Depth effects by species for each Site*Year. Model parameter estimates were converted from log odds using the formula $(\exp(\text{coefficient})-1)*100$ and are here reported on a percentage basis. Negative values indicate a % decrease where positive values represent a % increase in odds of emergence for a one increment increase in depth (~1 cm). Seeding depths were 1.4 cm, 2.6 cm, 3.6 cm, and 4.0 cm. For AGGR at Orovada in 2013 increasing seeding depth from 1.4 to 2.6 cm resulted in an 81.8% decrease in the odds of emergence. For AGHE2 at Wells in 2013 increasing seeding depth from 2.6 to 3.6 cm resulted in a 29.6 % increase in the odds of emergence. Emergence odds are estimated linearly across the range of depths, so the reported effect is the same between any two depths. Blank cells indicate inadequate seedling emergence, precluding computations.

Species	Trend	Frequency	Location and Year					
			Orovada 2013	Wells 2013	Ftn Green 2013	Orovada 2014	Wells 2014	Ftn Green 2014
			Depth effect (- % decrease, + % increase)					
ACMI	Negative	1				-23.7		
AGGR	Mixed -	3	-81.8 ***	-12.4		13.1		
AGHE2	Mixed +	2		29.6 ***	-4.6			
ARMAF	Mixed	2		-0.7	2.5			
ASFI	Mixed -	2		10.7 ***			-77.5	-163.2 ***
BAHO	Positive	4	3.1 ***	8 ***		12.2	52.5 **	
ENNU	Negative	3	-3.6	-12.3	-36.4 ***			
ERUM	Mixed +	3		175.8 ***	-20.6	118.3 ***		
HEMUN		0						
IPAG	Mixed -	2		-15.6 ***		18.2		
LONU2	Negative	6	-26.4 ***	-19.5 ***	-11 *	-13.9	-31.1 ***	
LUAR3	Mixed +	5	96.9 ***	24.8 ***		51.6 ***	-31.7	-9
LUPR2	Mixed +	5	102.1 ***	16.1 ***	160.6 ***	-23.8	-16.8	
MACA		0						
PEAC	Positive	1		2.8 ***				
PEPA6	Negative	3	-26.4	-24.7	-44.8 ***			

PESP	Mixed	4	20 **	-8.7 *	-8.2	0.5		
SPGR	Mixed +	3			-24.9 *	40.7 *		110.3 ***
SPMU	Positive	3			8.7 *	123.6 **		86.9 ***
STPII	Negative	1			-66.4 ***			

Table 2-5. F and P values for testing model predictors for 20 native forb species.

Model	Source	F statistic	P value
Global Model	Site	1596.33	<.0001
	Year	3823.38	<.0001
	Treatment	268.44	<.0001
	Species	647.44	<.0001
	Depth	4.32	0.03429
	Treatment*Species	21.28	<.0001
	Treatment*Site	280.06	<.0001
	Species*Site	87.17	<.0001
	Treatment*Year	197.65	<.0001
	Site*Year	250.4	<.0001
	Species*Depth	27.02	<.0001
Orovada 2103	Species	172	<.0001
	Depth	13.86	<.0001
	Species*Depth	26.44	<.0001
Wells 2013	Treatment	12.51	<.0001
	Species	603.72	<.0001
	Depth	9.35	<.001
	Treatment*Species	15.17	<.0001
	Species*Depth	16.85	<.0001
	Treatment*Depth	5.53	0.0186
Fountain Green 2013	Treatment	3.74	0.0656
	Species	56.74	<.0001
	Depth	10.83	<.001
	Treatment*Species	20.84	<.0001
	Species*Depth	11.89	<.0001
	Treatment*Depth	46.91	<.0001
Orovada 2104	Species	25.35	<.0001
	Depth	1.24	0.2655
	Species*Depth	3.79	<.0001
Wells 2014	Species	29.64	<.0001
	Depth	23.62	<.0001
	Species*Depth	3.09	0.014
Fountain Green 2014	Treatment	3.27	0.0636
	Species	18.01	<.0001
	Depth	0.02	0.8739
	Treatment*Species	9.44	<.001
	Species*Depth	14.88	<.0001
	Treatment*Depth	3.13	0.0734

Table 2-6. Model parameter estimates were converted from log odds using the formula $(\exp(\text{coefficient})-1)*100$ and are here reported on a percentage basis. Values represent the % decrease in emergence in control plots compared to row cover plots. Blank cells and other Site*Year combinations lacked adequate data for comparisons. For all species the odds of emergence were lower, though not always significantly, in control plots compared to row cover plots at the Wells and Fountain Green sites in 2013.

		Location and Year	
		Wells 2013	Fntn Grn 2013
Species	Trend	Control compared to Row Cover	Control compared to Row Cover
ACMI			
AGGR	Negative	-10.2 ***	
AGHE2	Negative	-80.5 *	-94.9
ARMAF	Negative	-93.8 ***	-95.1
ASFI	Negative	-70.6	
BAHO	Negative	-53.3	
ENNU	Negative	-57.1	-96
ERUM	Negative	-11.9 **	-99.2 ***
HEMUN			
IPAG	Negative	-92.6 ***	
LONU2	Negative	-61.8 ***	-95.8 *
LUAR3	Negative	-62.4	
LUPR2	Negative	-64.6	-98.2 **
MACA			
PEAC	Negative	-89.3 ***	
PEPA6	Negative	-83.3 *	-93.7
PESP	Negative	-78.1 ***	-94.0
SPGR	Negative		-99.3 ***
SPMU	Negative		-99 ***
STPII	Negative		-96.9

Table 2-7. The cost to establish 100 plants is reported for row cover and open treatments. The table does not account for implementation or row cover costs. NSulate fabric is currently priced at \$0.45 m². Annual variations in seed prices will alter costs. This table estimates costs through seedling emergence and not plant establishment. Expect additional mortality between emergence and establishment to increase costs.

Scientific Name	Common Name	Seeds\gram	Emergence rate (%)	Price / kg	Cost per 100 plants	Treatment
Agoseris grandiflora	large-flower goat chicory	460	0.67%	\$187	\$6.05	Row Cover
			0.51%	\$187	\$7.94	Open
Balsamorhiza hookeri	hairy balsamroot	222	0.90%	\$99	\$4.95	Row Cover
			1.00%	\$99	\$4.48	Open
Enceliopsis nudicaulis	naked stem sunray	360	0.95%	\$165	\$4.83	Row Cover
			0.51%	\$165	\$8.95	Open
Eriogonum umbellatum	sulphur flower buckwheat	460	1.02%	\$143	\$3.04	Row Cover
			0.20%	\$143	\$15.33	Open
<i>Lomatium nudicaule</i>	barestem biscuitroot	125	8.65%	\$187	\$1.73	Row Cover
			5.19%	\$187	\$2.89	Open
<i>Lupinus argenteus</i>	silvery lupine	45	2.95%	\$143	\$10.79	Row Cover
			1.22%	\$143	\$26.03	Open
<i>Lupinus prunophilus</i>	hairy big leaf lupine	53	3.31%	\$143	\$8.17	Row Cover
			1.46%	\$143	\$18.48	Open
<i>Penstemon pachyphyllus</i>	thickleaf beardtongue	600	0.42%	\$77	\$3.09	Row Cover
			0.32%	\$77	\$4.02	Open
<i>Penstemon speciosus</i>	royal beardtongue	1141	2.11%	\$77	\$0.32	Row Cover
			0.78%	\$77	\$0.87	Open
<i>Sphaeralcea grossulariifolia</i>	gooseberryleaf globemallow	890	1.11%	\$143	\$1.45	Row Cover
			0.25%	\$143	\$6.52	Open
<i>Sphaeralcea munroana</i>	Munro's globemallow	1357	0.65%	\$143	\$1.63	Row Cover
			0.15%	\$143	\$7.19	Open

CHAPTER 3

SoilWeb Improves Accuracy of Collection Site Plant Identification Among Big Sagebrush Subspecies.

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ABSTRACT

Developing simple diagnostics to identify subspecies is key in the restoration of sagebrush ecosystems. Such diagnostics are necessary at both the field level, aiding commercial seed collectors, as well as the certification level, ensuring accurate seed lot identity. Study objectives addressing these needs were two fold, first to evaluate the Web Soil Survey (<https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>) using the SoilWeb app as a tool to identify big sagebrush (*Atemisia tridentata*) subspecies in the field and second, to develop a model to best distinguish subspecies identity of harvested sagebrush seed. We found the SoilWeb app to be an accurate and informative tool to identify the subspecies of big sagebrush stands. Where county level soil data was available, the SoilWeb app proved 100% accurate in identifying the dominant big sagebrush subspecies on site. Richardson et al. (2015) proposed the first techniques to validate subspecies identity using seed. We found *wyomingensis* mean seed weights were 30% lighter than those reported by Richardson et al. (2015). The Richardson et.al. (2015) seed weight criteria was ineffective at categorizing our seed collections to the correct subspecies; only classifying 19% correctly. To improve upon this, we combined data from our study and the Richardson et al. (2015) study to develop a multifactor modeling approach using

recursive partitioning and classification trees, emphasizing factors common to both data sets and also obtainable from source identified commercial seed lots. The classification tree correctly classified 80% of 2x *tridentata* sites but just 45% of *wyomingensis* sites. Currently, the best means to identify *wyomingensis* sagebrush seed lots is to germinate seed and assign identity based on the cytotype and UV florescence of those plants.

INTRODUCTION

Large quantities of big sagebrush (*Artemisia tridentata*; hereafter just sagebrush) are seeded in restoration efforts in the Interior West, often with low establishment success (Knutson et al. 2014). The current emphasis on locally adapted seed and the appropriate pairing of seed source to restoration site (Oldfield and Olwell 2015) has highlighted the ecological differences of sagebrush subspecies (Goodrich et al. 1985) and implicated incorrect matching of subspecies to site as a primary reason for seeding failure (Dumroese et. al. 2015). Big sagebrush subspecies (*Artemisia tridentata* ssp. *tridentata*, *A.t.* ssp. *vaseyana*, and *A.t.* ssp. *wyomingensis*), hereafter referred to as *tridentata*, *vaseyana* and *wyomingensis*, respectively, make up the majority of the restoration seed market. Sagebrush seed is certified and marketed by subspecies, yet there have been no techniques to validate the subspecies identity of seed itself. Rather, seed lot identity has been based on the subspecies identification of plants present at the collection site along with harvest date and characteristics of chaff harvested with the seed, including odor and leaf morphology (Tilley et al. 2006).

Richardson et al. (2015) proposed the first techniques to validate subspecies identity from seed. Using a simple protocol combining the existing knowledge of subspecies plant and leaf morphology (Rosentreter 2004, Welsh et.al 2003) and UV reflectance patterns (Stevens and

McArthur 1974, Shumar 1982, Rosentreter 2004) with a proposed novel protocol (seed weight), Richardson et al. (2015) reported that big sagebrush seed could be accurately identified to the subspecies level. Based on these techniques, they assessed the composition of 30 commercial seed lots purchased by the Bureau of Land Management between 2013 and 2014, source identified to subspecies (UCIA Certified Wildland 2017) and reported that routinely what is on the tag is not what is in the bag. Due to the heterogeneous distribution of sagebrush subspecies and inter-subspecies hybridization (Freeman et al. 1991, McArthur et al. 1988) caused by landscape and soil heterogeneity (i.e., ecotones), it is expected that large seed collections will be of mixed composition but primarily composed of the intended subspecies. However, based upon these new criteria, one of five *tridentata* lots, one of seven *vaseyana* lots and 15 of 18 *wyomingensis* lots (83%) were apparently misidentified. Most restoration seedings occur in habitat occupied by the *wyomingensis* subspecies, thus having a high proportion of lots of this subspecies misidentified would be of serious concern to restoration practitioners.

Developing simple diagnostics to identify subspecies is key in the restoration of sagebrush ecosystems. Such diagnostics are necessary at both the field level, aiding commercial seed collectors, as well as the certification level, ensuring accurate seed lot identity. Study objectives addressing these needs were three fold. The first, was to evaluate the Web Soil Survey (<https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>) (Soil Survey Staff 2018) using the SoilWeb app as a tool to identify subspecies of sagebrush in the field (Table 3-1 and Figure 3-1). The SoilWeb app accesses county level soil surveys in digital format via smartphone providing detailed soil, and of primary interest to our study, vegetation data, tied to a user's geographic location. Location-specific ecological site descriptions identify the primary sagebrush subspecies present and similar data from adjacent map units provides information about the potential

abundance of other sagebrush subspecies. The second was to test the accuracy of the Richardson et al. (2015) technique to characterize subspecies stand identity at our sites. The third objective was to evaluate if the seed lot identity can be improved through classification tree modeling and adding physiological, morphological and geographic variables to seed weight.

METHODS

To evaluate the SoilWeb application utility, to obtain seed samples for testing Richardson et al. (2015) protocols and to develop improved seed identification models, we collected seed at 14 *wyomingensis* sites in 2017. Two other sampled populations were laboratory identified as *tridentata* and *vaseyana* big sagebrush sites (Figure 3-2). Data from these populations, was included in classification and modeling efforts. Sites represent a range in geographic and environmental variation and included: 1) northern Malheur County, Oregon, 2) northern Nevada between Salt Lake City, Utah and Winnemucca, Nevada and 3) Beaver County, Utah west of Milford.

At eight sites where county level soils data were available, we accessed SoilWeb on site using a smart phone or off site (post sampling) using the web application where cell service was not available. The ecological site description for each map unit identified the dominant sagebrush subspecies assigned to each sampling area (Table 3-2, Dominant Sagebrush) as well as an estimate of the percentage of land within the land association (mapped polygon) occupied by this and other sagebrush subspecies (Table 3-2, Map Unit Composition, Dominant Sagebrush). Individual plant subspecies identity was scored in the field then verified with lab techniques, described below. Field identification relied on the ecological site description obtained from the SoilWeb app. Individual study plants were assigned to the dominant subspecies unless

morphological or phenological characteristics suggested that the plant might be more appropriately classified as a different subspecies following Rosentreter's key (2004). At sites where SoilWeb data was lacking, subspecies designation was determined by identifying plants according to Rosentreter's key (2004). At each site, plant and site data as well as leaf tissue and seed were collected from 10 to 30 plants. Individual plants were spaced 30-m apart along one or more linear transects. Oregon populations (n=7) ranged in elevation from 821-1425 m and were harvested between November 21st and 30th, 2017. Northern Nevada populations (n=7) ranged in elevation from 1360-1740 m and were harvested November 7th to 11th, and Beaver County, Utah populations (n=2) occurred at 1550 and 1660 m and were harvested November 2nd. At these dates, seed was beginning to shatter at all but two Nevada sites. Seed samples were harvested by placing clipped inflorescences from individual plants in paper bags. Prior to cleaning, samples were air dried at 25° C. Seed was cleaned to high purity using soil sieves (45 µm, 325 mesh), hand screening and a Hoffman South Dakota seed blower. Four replications of 50 seeds were randomly sampled from the cleaned seed lots and weighed using an analytical scale (0.1 mg readability and repeatability).

Laboratory techniques to identify individual plant subspecies relied on a combination of ultraviolet reflectance score and cytotype. To characterize cytotype as diploid (2x) or tetraploid (4x), ploidy level was determined from fresh leaf tissue using a Partec Cyflow flow cytometer (see Richardson et al. 2012). Leaf tissues were also fluoresced and given a reflectance score (UV) by placing chopped leaf material in small, glass vials filled with water, and then viewed under black light after several minutes (Stevens and McArthur, 1974). Score classification options were: intense (5), strong (4), moderate (3), light (2) and colorless (1) (Rosentreter 2004). For a complete characterization of laboratory identified subspecies categories refer to Table 3-3.

We treated laboratory results as definitive and compared field identification of sagebrush plants to lab identification to assess field accuracy.

For each site, we estimated stand composition based upon the proportion of study plants of the dominant subspecies (as identified with lab techniques) compared to the total number of plants sampled. For example, at the Ryndon, NV site, laboratory techniques identified 11 of the 12 sampled plants (92%) as *wyomingensis* (Table 3-2). We calculated field accuracy as the proportion of plants where field and lab identification were the same for each site. For example, at Silver Zone, NV 77% of plants were both field and lab identified as *wyomingensis*.

In the Richardson et al. (2015) study, seed samples were collected from native populations and from first-generation progeny of those populations growing in common gardens. Where complete replication was available, seed was collected from five plants at each native population site and from two plants from each population growing in common gardens. For each plant, three (native populations) or six (common gardens) subsamples of 10-count seed were weighed. More recently, Richardson (pers. comm.) developed big sagebrush subspecies ID seed testing protocols adapted for use by seed certification laboratories that are consistent with the Association of Official Seed Certifying Agencies protocols. These protocols call for weighing eight replications of 100 seeds per lot. Being aware of the forthcoming increased sample size recommendations, in our study we calculated mean weights from four, 50-seed subsamples per plant. Fifty seeds were selected because about 30% of our samples lacked adequate seed numbers for four 100-count reps but only 15% lacked adequate numbers for four 50-count reps. By selecting 50-seed count reps, 85% of our seed samples were retained. For comparison to the Richardson et al. (2015) data, our 50-seed count weight data were converted to 10-count weights by dividing by five.

For 2017 data, we analyzed seed weight differences among subspecies using a generalized linear model (GLM) with family set to Gamma. Post hoc analysis used Tukeys HSD (multcomp package, Hothorn et al. 2008) to find means that were significantly different ($p=0.05$) (Table 3-4). Confidence intervals (99%) (Table 3-5) were computed using the predictor effects function (Fox 2003, Fox and Weisberg 2018, Fox and Weisberg 2019) following the GLM model. A primary objective was to compare *wyomingensis* mean seed weights between the Richardson et al. (2015) data and our data (Table 3-5). For this, we used a T test constructed using mean, standard deviation and sample size. We then assessed the accuracy of using Richardson et al. (2015) mean seed weight classification criteria for our dataset by computing the percentage of sites correctly classified to subspecies. To do this we compared mean seed weights from each site with Richardson et al.'s (2015) confidence intervals and computed the % correctly classified. Data from plants not of the primary subspecies (lab identified) at each site were removed so the comparison was exclusive to pure subspecies seed lots.

Our next objective was to determine if a more accurate characterization of subspecies seed identity could be developed using recursive partitioning and regression tree modeling (rpart, Therneau and Atkinson 2018). For this analysis, data from both studies were combined into a single dataset. The combined data set contained the following variables: study, subspecies, 10 - count (or 50 to 10-count converted) seed weight, seed ripeness, UV score, latitude, longitude, and elevation. This type of analysis requires a large dataset so that classifications are not artifacts of sampling deficiencies. Then we used recursive partitioning and classification trees as a method of mean separation to classify individual plant seed samples into subspecies groups. We ran iterations of models beginning with 6 variables and assessed appropriateness of nodes and model accuracy. As deficiencies were identified offending elements were removed. We removed

2x and 4x *vaseyana* and 4x *tridentata* from the dataset when nodes created breakpoints based on inadequate geographic sampling. Lacking *vaseyana* populations, UV was no longer relevant. Longitude was removed due to inadequate *wyomingensis* sampling on the western portion of the species distribution. The final dataset contained 16 populations of 2x *tridentata* and 26 populations of *wyomingensis*. Seventy-five % of the data was used to train the models with 25% retained to test the models. All data analyses were run in the R statistical package (R Core Team 2018). We selected the model most accurate at assigning subspecies from modeled variables. This assessment is based on running individual plant seed weight subsamples with latitude and elevation corresponding to collection location through the model and assigning a subspecies category. For the classification tree to be useful as a certification tool it needs to draw on site level data rather than individual plant data. For final model evaluation, we used mean site level data for seed weight, elevation and latitude to classify all *tridentata* and *wyomingensis* sites from both studies to subspecies.

RESULTS

At the eight sites where SoilWeb data was available, *wyomingensis* was listed in the ecological site descriptions of prevalent soil map units as the dominant subspecies. Laboratory plant identification validated that these stands were dominated by *wyomingensis* plants, for 100% correct stand classification (Table 3-2). At the eight sites where soil web data was not available, six sites were correctly field identified as *wyomingensis* while two sites (one *tridentata* and one *vaseyana*) were incorrectly classified as *wyomingensis*.

We observed variation in stand composition across the geographic area sampled, with more mixed species stands among Oregon populations. At four locations, stand composition was 100%

true to stand identity (all plants were identified as the same subspecies) while stands of mixed subspecies composition were present at 75% of the locations. Within stands, nondominant subspecies or hybrid occurrence was low at just 11% (Figure 3-3). Stand composition ranged from 71% to 100% true to stand identity.

To assess our ability to correctly identify plants in the field, we compared the field identification of each plant to its corresponding lab identification. Where SoilWeb data was not available (Table 3-2) field identification matched laboratory identification with 72% accuracy. Where SoilWeb data was available field identification matched laboratory identification with 91% accuracy. Hybrids made up 4% of the samples and were always incorrectly identified. These plants were morphologically indistinguishable from nonhybrids but had higher than expected UV scores. Five % of plants were field classified as *tridentata* but lab classified as *wyomingensis*. Four % of the plants were field classified as *wyomingensis* but lab classified as *tridentata* and 2% of the plants were field classified as *wyomingensis* but lab classified as *vaseyana*.

Mean seed weights observed in our study were significantly lower than those reported by Richardson et al. (2015) (Table 3-5). *Wyomingensis* mean seed weights were 30% lighter. To assess the accuracy of mean seed weight-based subspecies identification we compared mean seed weights at each site with Richardson et al.'s (2015) confidence intervals and computed the % correctly classified. Mean seed weights at two *wyomingensis* and one *vaseyana* site fell within mean weight criteria. The remaining 12 *wyomingensis* and one *tridentata* sites were lighter than subspecies specific weight criteria (Table 3-6), for a classification accuracy rate of 19%.

Because of poor classification accuracy using the single factor, seed weight, our focus turned to determining if multiple factor classification would offer better results. Our best classification

tree included the variables seed weight, latitude and elevation. It was 86% accurate at assigning holdout or test data from individual plant samples to the correct subspecies categories. The usefulness of the model is however, based on its ability to classify sites to the correct subspecies, using mean site data rather than individual plant samples. In this regard, the classification tree correctly classified 12 of 15 (80%) *2x tridentata* sites but just 10 of 22 (45%) *wyomingensis* sites (Figure 3-4).

DISCUSSION

In our study, we found the SoilWeb app to be an accurate and informative tool to identify sagebrush stands. It provides field going personnel rapidly accessible information in a format which, with minimal experience, addresses the primary question for seed collectors; what sagebrush subspecies is this? Where data was available, SoilWeb proved 100% accurate in identifying the dominant sagebrush subspecies on site. There are several limitations to the tool's application. Its use should be restricted to native sagebrush sites, as restoration plantings likely used seed of different origin. Soil coverage gaps limited the tool's usefulness in some regions. Cellular coverage gaps also limited the tool to an extent. However, this was overcome by accessing the same information via the Web Soil Survey from a personal computer after a sagebrush stand of interest had been identified and its GPS coordinates recorded. The distribution of sagebrush land associations extends beyond those evaluated in this study, to include many others. Based on this study, we anticipate a high degree of accuracy across untested associations with their corresponding ecological site descriptions. However, to best extend the use of this tool, its ability to accurately identify the correct sagebrush subspecies should be tested across a wider variety of sites and stands of subspecies than we did in this study.

To assess the likelihood of harvesting mixed subspecies seed lots, we evaluated stand composition and field accuracy at each site. Laboratory plant identification data, based on UV reflectance scores and cytotype were compared to field identity as the basis for these comparisons. Stands of mixed subspecies composition were common (12 of 16), although the occurrence rate of plants of nondominant subspecies was low in our seed collections (21 of 191). Some (n=7) of the nontarget plants displayed traits supporting a hybrid origin (typically introgression of *wyomingensis* and *vaseyana*) occurring in *wyomingensis* dominated sites. Mean seed weights of these hybrids were significantly heavier (p=0.027) than those from *wyomingensis* plants. Based on their low frequency and likely similar habitat adaptation, inclusion in a commercial seed lot is of little concern. The larger issue is *tridentata* or *vaseyana* subspecies mixed with *wyomingensis*. In our study, we found eight of 14 sites of *wyomingensis* dominated stands with intermixed seed ranging from 5 to 28 percent with a mean of 17 percent. (Figure 3-3). Similarly, such a low frequency of occurrence is of little concern.

Field classification correctness or field accuracy is a portrayal of seed collector's ability to accurately field identify plants and thereby harvest a seed lot composed primarily of the intended subspecies. We correctly identified plants 91% of the time where SoilWeb data was available to inform species composition. At four of eight sites, 100% of plants were correctly identified. Where no SoilWeb data was available we correctly identified plants 72% of the time. This included two sites with 20% and 30% field accuracy where the prominent subspecies was incorrectly identified and one site where field accuracy was 100%. Our sample plots were situated in the heart of the dominant subspecies and, while we attempted to capture variability by spacing sample plants at 30-m intervals, our sample footprint is small, particularly in comparison to the acreage necessary to harvest commercial seed quantities. The opportunity to cross

subspecies boundaries or encounter hybrids increases with harvest acreage and is in reality of greater concern than represented by our data. Based on this experience, harvesting a seed lot composed largely of the intended subspecies, is most likely, where Soil Web data is used to initially identify the dominant subspecies, followed by restricting seed collection to soil map units of the intended subspecies and excluding harvest of field identified nontarget plants.

Richardson et al. (2015) demonstrated that seed weights varied among subspecies, and of particular value, *tridentata* seed was significantly lighter than *wyomingensis* seed. Unexpectedly, we found *wyomingensis* mean seed weights were lighter than Richardson et al.'s (2015) weight intervals (Table 3-5). To verify that seed weight differences between the studies were real, we reviewed processing and weighing protocols and seed harvesting methods between the studies. Seed harvesting methods varied. For our study collections and original collections in the Richardson et al. (2015) study, seed was harvested by cutting inflorescences from plants. At common gardens in the Richardson et al. (2015) study, inflorescences were bagged and bags were collected following natural seed shatter. We speculate that allowing seed to mature and naturally shatter may result in heavier seed. Richardson et al. (2015) reported a 0.18 mg environmental effect difference in mean seed weight between original collections and garden sites (their Figure 3-4) that may partially be attributable to seed harvest differences. His study was not designed to address this question directly and data did not permit a precise comparison. In the present study, seed ripeness was classified as 25% shatter, full shatter, or pre-shatter and requiring cutting when harvested. We detected no differences in seed weights between shatter categories for *wyomingensis* ($p=0.49$) or *tridentata* ($p=0.32$). Because our harvest techniques and timing more closely follow commercial seed collector practices, the seed weights from our study

and Richardson et al.'s (2015) original collections should better reflect those expected for commercial seed collections.

The Richardson et.al. (2015) seed weight criteria was ineffective at categorizing our seed collections to the correct subspecies, only classifying 19% correctly. Subsequent efforts to develop a multifactor classification tree, using a larger-combined dataset, also failed to meet the primary objective, that of distinguishing *wyomingensis* populations. However, with 45% classification accuracy for *wyomingensis* and 80% classification accuracy for 2X *tridentata*, the current approach, using just 22 and 15 populations respectively, suggests classification trees have potential, if acceptable accuracy can be attained with additional sampling. Inadequate sampling of *vaseyana* required removing this subspecies and nonsymmetrical geographic sampling among *tridentata* and *wyomingensis*, where distribution overlaps are known to occur, resulted in artificial node break points rather than real subspecies distribution constraints. Most drastically this resulted in removal of longitude due to inadequate *wyomingensis* sampling on the western portion of the species distribution. It may be, that broadening the geographic extent of the study to cover the entirety of each subspecies distribution, or at least ensuring each species is represented at similar locations where overlapping geographic distribution exists, would permit inclusion of removed variables (UV and Longitude) and subspecies and cytotype groups (2X and 4X *vaseyana*, and 4X *tridentata*) leading to a more elegant model with improved accuracy.

A significant challenge in distinguishing among subspecies resulted from seed weights being significantly different between studies. These differences suggest either environmental factors have an effect on seed weight aside from genetic controls, or inadequate temporal and spatial sampling occurred in one or both studies to accurately represent seed weights. Busso (2005) reported mean sagebrush seed weight varied by a factor of 1.4 between locations and years and

that monthly precipitation explained 85% of the variation in seed weight. *Wyomingensis* seed weights between the Richardson et al. (2015) and our study similarly varied by a factor of 1.4 (Table 5). Caveats to Busso's (2005) study include *wyomingensis* plants were selected based on visual similarity rather than more definitive means such as UV reflectance and cytotype and sampling transects were up to 10 km long and 390 m in elevational gradient increasing odds of encountering different subspecies. There is evidence of environmental effects on seed weight in crop species. Drought or heat stress have been shown to lower individual seed weights in lentils (*Lens culinaris*) (Sehgal et al. 2017), wheat (*Triticum*) (Gooding et al. 2003), rice (*Oryza*) (Britz et al 2007), and wild sunflower (*Helianthus annuus*) (Hernandez, Poverene and Presotto 2018), while growth season and location effected seed weight of beans (*Phaseolus vulgaris*). Whether small seeded sagebrush may respond similarly to these large seeded crop species is unverified. Richardson et al.'s (2015) common gardens were sited in locations with dramatically different elevation (974 m and 2105m) and precipitation (224 mm and 414 mm) yet they report environmental effects on seed weight being ≤ 0.27 mg and posit plant competition as a possible explanation. Across both plant and animal kingdoms, Dani & Kodandaramaiah (2017) report that, while offspring size is generally less variable than offspring number, variation in offspring size increases under sub-optimal environmental conditions. Additional study will be required to definitively determine if and to what degree environmental and other conditions affect sagebrush seed weight.

Alternatively, sampling deficiencies may be the cause of observed seed weight differences between studies. Richardson et al.'s (2015) sampling strategy was geographically broad, extending across much of the distribution of big sagebrush, but *wyomingensis* samples were largely vacant from the Great Basin region where sagebrush is most often used in rangeland

seedings. Data for the present study was much more geographically restrictive but in areas not previously sampled. Differences between studies may suggest that geographic variability in seed weight may be more complex than represented by either study alone.

MANAGEMENT IMPLICATIONS

To improve composition of commercial seed lots the SoilWeb app proved to be a valuable tool aiding field identification of sagebrush. At sites where map coverage was available, the SoilWeb app was 100% accurate in identifying the primary sagebrush subspecies. Where sagebrush stands consist of multiple intermixed or adjacent species seed collecting companies and wildland seed certification entities can use the app to understand the distribution and abundance of other subspecies in similar and adjacent soil components. Lastly, post-harvest lot identity should not be based on seed weight. The range in seed weights among sagebrush subspecies is too variable to permit distinguishing among subspecies based on this criterion alone and based on existing data, in combination with latitude, longitude and elevation. Currently, the best means to definitively identify sagebrush seed lots is to germinate seed and assign identity based on the cytotype and UV florescence of those plants (Table 3-3).

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FIGURES

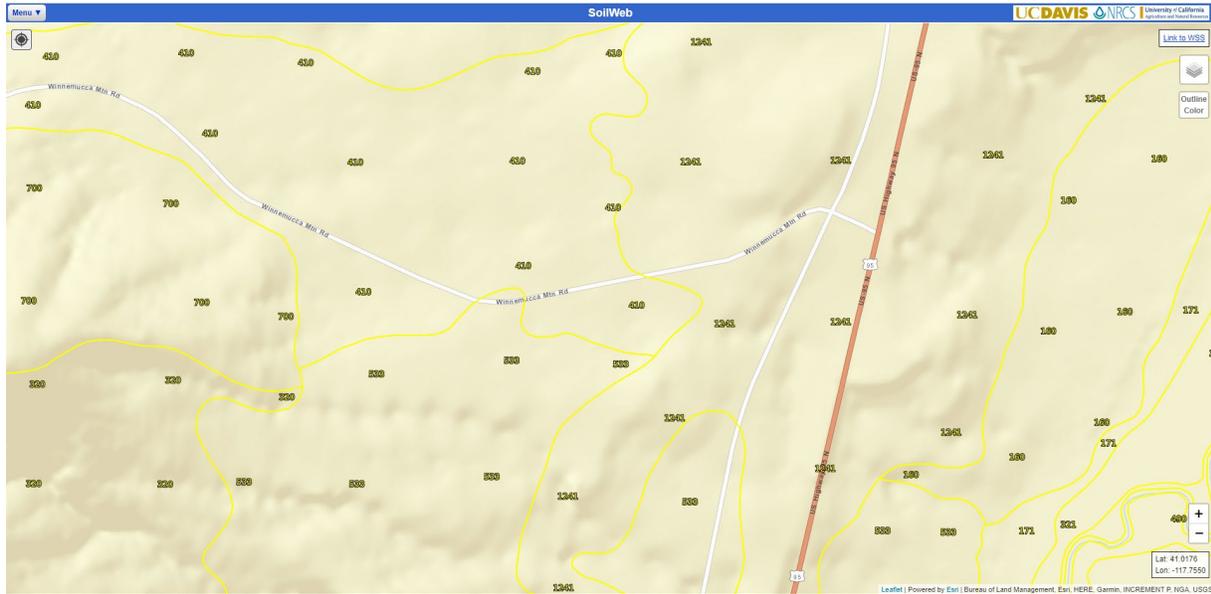


Figure 3-1. Soil associations are outlined in yellow polygons in this demonstration map of a *wyomingensis* seed collection area (Table 3-1). The map area is near Winnemucca mountain road, on the west side of US-95, 5.5 kilometers north of Winnemucca, NV.

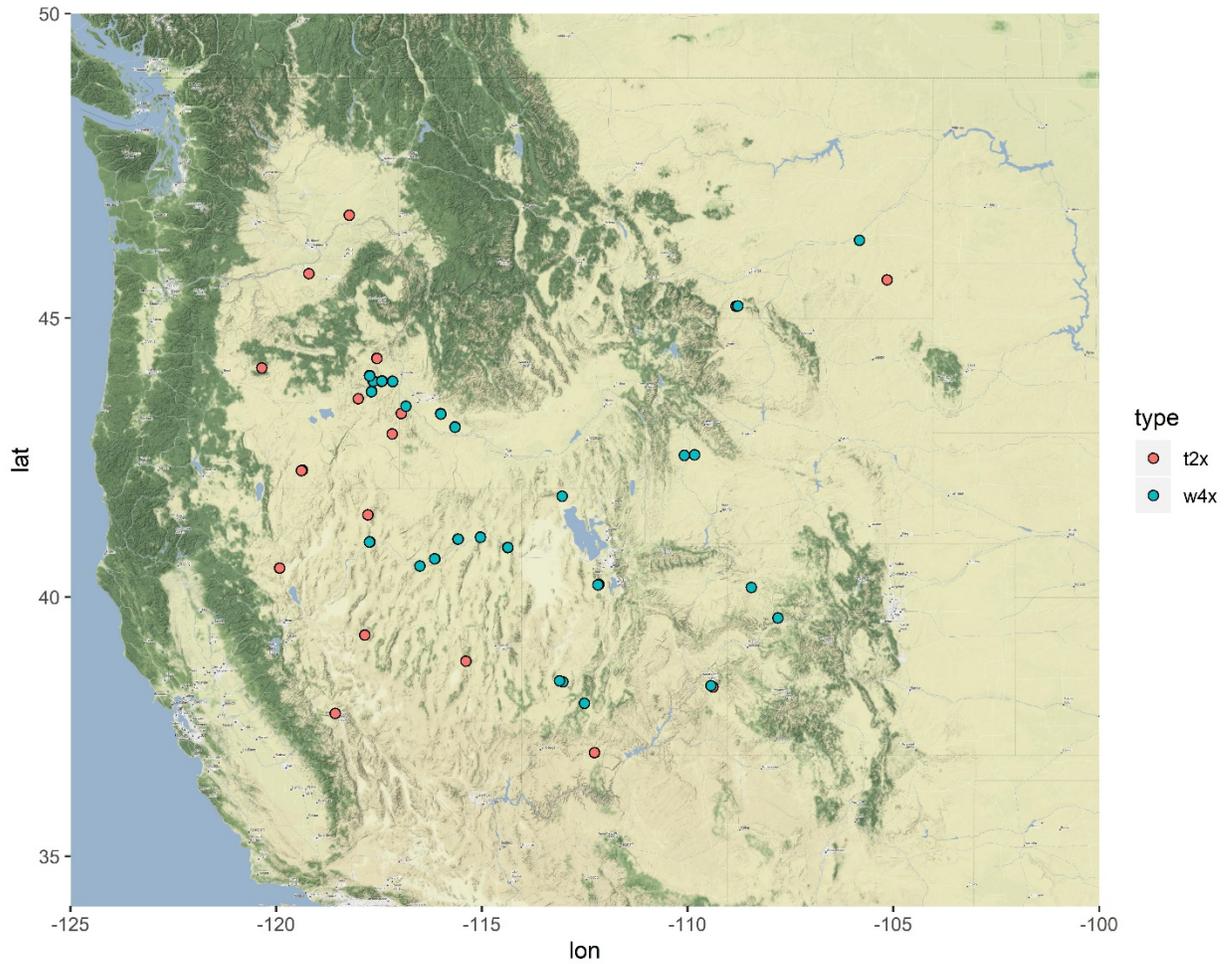


Figure 3-2. Distribution of *tridentata* (t2x) and *wyomingensis* (w4x) populations represented in these studies.

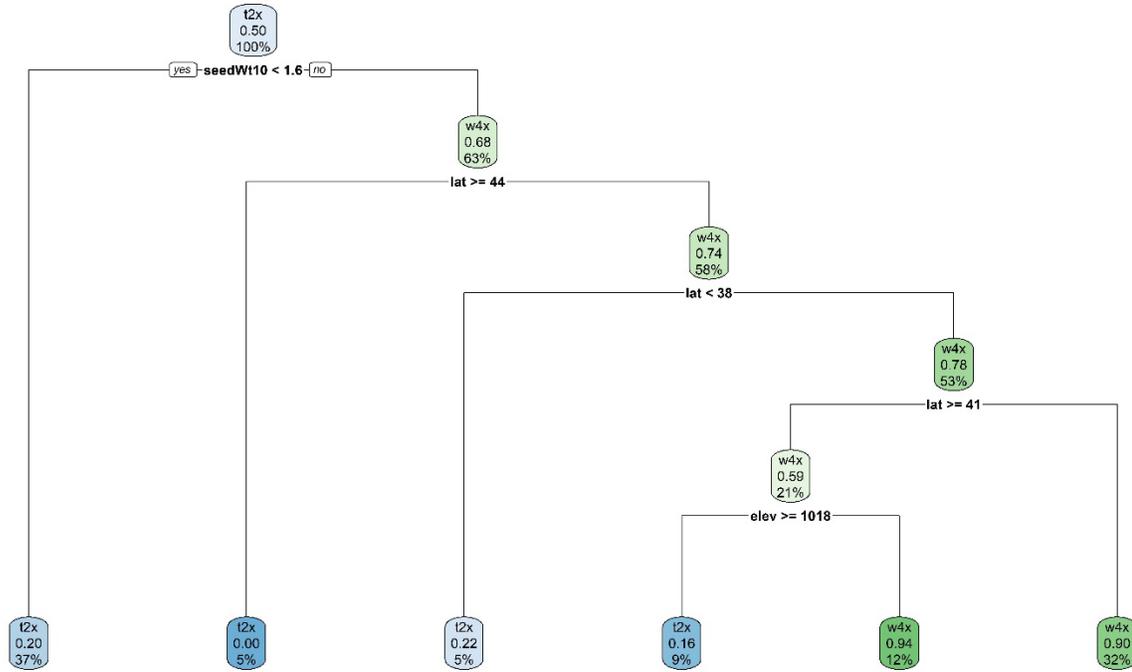


Figure 3-4. Our best classification tree included the variables seed weight, latitude and elevation. The classification tree functions as a sequential flow chart with yes or no decisions made at each node. Yes responses lead to the left while no responses lead to the right. It was 86% accurate at assigning test data from individual plant samples to the correct subspecies categories. The usefulness of the model is however, based on its ability to classify sites to the correct subspecies, using mean site data rather than individual plant samples. In this regard, the classification tree correctly classified 12 of 15 (80%) *2x tridentata* sites but just 10 of 22 (45%) *wyomingensis* sites.

TABLES

Table 3-1. This table serves as an example of how to use SoilWeb to identify appropriate sagebrush stands for a *wyomingensis* seed collection. Here we've chosen a sagebrush stand near Winnemucca mountain road, on the west side of US-95, 5.5 kilometers north of Winnemucca, NV, USA (41.0239, -117.7117) (also see Figure 3-1). Five soil associations occur within our area of interest. Individual soil components are listed for each soil association along with the percentage of the map unit they occupy. By selecting each component and viewing the Ecological Site Description under the Land Classification tab, the dominant shrub species is identified. Soil map unit 1241 consists of two soil components, Laped which makes up 50% of the unit and Boger that makes up 35%. In Laped the dominant shrub is *Atriplex confertifolia* while *wyomingensis* dominates in Boger. Notice, sagebrush is only identified to species rather than subspecies in map unit 410. Collecting in this unit will first require stand identification using in field diagnostics. Using table information to further inform seed collecting, we observe *wyomingensis* is the primary sagebrush species in map unit 533. Unit 700 is dominated by *A. nova* and *vaseyana* and should be avoided. May area 320 is composed of 45% *vaseyana* and 40% *wyomingensis*. Making a *wyomingensis* seed collection in this unit will require distinguishing among these subspecies.

Soil Association	Laped-Boger association (1241)		Orovada-Bliss association (410)		Shabliss-Connel association (533)		Atlow-Gowjai association (700)		Havingdon-Burrita association (320)	
	▲ Map Unit Composition	Dominant shrub	▲ Map Unit Composition	Dominant shrub	▲ Map Unit Composition	Dominant shrub	▲ Map Unit Composition	Dominant shrub	▲ Map Unit Composition	Dominant shrub
Component	50% - Laped	<i>Atriplex confertifolia</i>	45% - Bliss	<i>Artemisia tridentata</i>	60% - Shabliss	<i>A.t. wyomingensis</i>	50% - Atlow	<i>Artemisia nova</i>	45% - Havingdon	<i>A.t. vaseyana</i>
Component	35% - Boger	<i>A.t. wyomingensis</i>	45% - Orovada	<i>Artemisia tridentata</i>	25% - Connel	<i>A.t. wyomingensis</i>	35% - Gowjai	<i>A.t. vaseyana</i>	40% - Burrita	<i>A.t. wyomingensis</i>

Table 3-2. Data obtained from the Soil Web app for each seed collection location. Map polygons represent land associations and their associated soil map units. The ecological site description identifies the prevalent sagebrush subspecies within each soil map unit. The number of sampled plants included in analysis is listed as n. Field accuracy is the proportion of plants where field identification and lab identification were the same. Stand composition is the proportion of plants of the dominant subspecies at each site compared to the total number of plants sampled.

Location	Land Association	Map Unit Composition	Ecological Site Description	Dominant Sagebrush	n =	Field Accuracy	Stand Composition
Beowawe, NV 40.579, -116.5079	Malpais-Rock outcrop-Rubble (MA)	40% - Malpais 30% - Rock outcrop 15% - Rubble land	Loamy 8-10 P.Z.	<i>A.t.wyomingensis</i>	5	100%	100%
Beverly Hills, NV 41.1085, -115.03919	Dacker-Nevador-Kelk association (231)	45% - Dacker 25% - Nevador 20% - Kelk 4% - Hunnton 3% - Oupico 3% - Enko	Loamy 8-10 P.Z. Loamy 8-10 P.Z. Loamy 8-10 P.Z. Loamy 8-10 P.Z. Loamy 8-10 P.Z. Loamy 8-10 P.Z.	<i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i>	9	89%	89%
Five Mile, UT 40.2298, -112.1859	Hiko Peak-Taylorsflat complex (24)	45% - Hiko Peak 40% - Taylorsflat 5% - Spager 5% - Medburn	Semidesert Gravelly Loam Semidesert Loam Semidesert Shallow Hardpan (8-10 Ppt) Semidesert Shallow Hardpan (8-10 Ppt)	<i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>A. nova</i> <i>A. nova</i>	10	100%	100%
	Linoyer very fine sandy loam(LaC)	85% - Linoyer 15% - Unnamed soils	Semidesert Sandy Loam	<i>A. t. wyomingensis</i>			
Milford, UT 38.4020, -113.0362	Crestline sandy loam(167)	80% - Crestline 10% - Hiko Peak 5% - Heist 3% - Snake Hollow 2% - Petrocalcids	Semidesert Loam Semidesert Gravelly Loam Semidesert Sandy Loam Upland Gravelly Loam Semidesert Shallow Hardpan	<i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>A. t. bonnevillensis</i> <i>A. nova</i>	11	91%	91%
Ryndon, NV 41.0732, -115.5824	Kelk-Kelk, occasionally flooded-Enko(141)	60% - Kelk 15% - Kelk 15% - Enko 4% - Sonoma 3% - Wieland 3% - Bloor	Loamy 8-10 P.Z. Dry Floodplain Loamy 8-10 P.Z. Dry Floodplain Loamy 8-10 P.Z. Saline Bottom	<i>A. t. wyomingensis</i> <i>A. t. tridentata</i> <i>A. t. wyomingensis</i> <i>A. t. tridentata</i> <i>A. t. wyomingensis</i>	12	100%	92%
Silver Zone, NV 40.9175, -114.3729	Threese-Tosser association (1410)	65% - Threese 20% - Tosser 8% - Okan 4% - Pyrat 2% - Heist 1% - Kunzler	Loamy 8-10 P.Z. Shallow Calcareous Slope 8-10 P.Z. Droughty Loam 8-10 P.Z. Loamy 8-10 P.Z. Coarse Silty 6-8 P.Z. Sodic Terrace 8-10 P.Z.	<i>A. t. wyomingensis</i> <i>A. nova</i> <i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i>	22	77%	95%
West Carlin, NV 40.7130, -116.1485	Cherry Spring-Berning association (CF)	70% - Cherry Spring 20% - Berning	Loamy 8-10 P.Z. South Slope 8-12 P.Z.	<i>A. t. wyomingensis</i>	10	100%	100%
Winnemucca, NV 41.0250, -117.7273	Laped-Boger association (1241)	50% - Laped 35% - Boger	Loamy 5-8 P.Z. Loamy 8-10 P.Z.		26	92%	92%
	Shabliss-Connel association (533)	60% - Shabliss 25% - Connel	Loamy 8-10 P.Z. Droughty Loam 8-10 P.Z.	<i>A. t. wyomingensis</i> <i>A. t. wyomingensis</i> <i>Artemisia tridentata</i>			
Big Wash, UT	38.4243, -113.1121	No Digital Data Available			12	75%	75%
Bonita, OR	44.0060, -117.7360	No Digital Data Available			14	86%	93%
Crowley, OR	43.7238, -117.6829	No Digital Data Available			10	100%	90%
Harper Westfall, OR	43.9079, -117.6527	No Digital Data Available			14	71%	79%
Lytle, OR	43.89777, -117.1681	No Digital Data Available			7	86%	71%
Shumway, OR	43.5935, -118.0024	No Digital Data Available			5	20%	80%
Vines Hill, OR	43.8992, -117.4389	No Digital Data Available			14	79%	93%
Willow Creek, OR	44.30669, -117.5594	No Digital Data Available			10	30%	100%

Table 3-3. Plants were identified to subspecies based on combinations of UV and cytotype according to the following categories. Numbers in parenthesis represent the number of plants classified to that subspecies category. The single plant classified as a *tridentata* hybrid occurred at a *wyomingensis* dominated site and was diploid but with intermediate UV florescence. Plants classified as *wyoming* hybrids occurred at *wyomingensis* dominated sites but had higher UV florescence scores than expected.

UV	Cytotype	Subspecies category
1	2x	<i>tridentata</i> (19)
1	4x	<i>wyomingensis</i> (94)
2	2x	none
2	4x	<i>wyomingensis</i> (62)
3	2x	<i>tridentata</i> hybrid (1)
3	4x	<i>wyomingensis</i> hybrid (7)
4	4x	<i>vaseyana</i> (8)

Table 3-4. Multiple comparisons of big sagebrush mean seed weights across all 2017 sites using Tukey contrasts.

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
<i>tridentata</i> hybrid - <i>tridentata</i> = 0	-0.065640	0.102949	-0.638	0.9845
<i>vaseyana</i> - <i>tridentata</i> = 0	-0.406546	0.041600	-9.773	<0.001
<i>wyomingensis</i> - <i>tridentata</i> = 0	-0.353688	0.037164	-9.517	<0.001
<i>wyomingensis</i> hybrid - <i>tridentata</i> = 0	-0.415973	0.042031	-9.897	<0.001
<i>vaseyana</i> - <i>tridentata</i> hybrid = 0	-0.340906	0.098082	-3.476	0.0052
<i>wyomingensis</i> - <i>tridentata</i> hybrid = 0	-0.288048	0.096284	-2.992	0.0252
<i>wyomingensis</i> hybrid - <i>tridentata</i> hybrid = 0	-0.350332	0.098265	-3.565	0.0037
<i>wyomingensis</i> - <i>vaseyana</i> = 0	0.052858	0.020065	2.634	0.0699
<i>wyomingensis</i> hybrid - <i>vaseyana</i> = 0	-0.009426	0.028072	-0.336	0.9992
<i>wyomingensis</i> hybrid - <i>wyomingensis</i> = 0	-0.062284	0.020944	-2.974	0.0267

(Adjusted p values reported -- single-step method)

Table 3-5. Mean seed weights (mg) and associated 99% confidence limits are reported for this study and compared to Richardson et al. (2015) published data. T-tests were used to assess differences between means.

		Lower	Mean Weight	Upper	P value
Richardson et al data	<i>wyomingensis</i>	2.28	2.82	3.26	<0.001
Current study data	<i>wyomingensis</i>	1.93	1.98	2.03	

Table 3-6. Sagebrush sites sampled in 2017 are listed with the primary subspecies present and the number of seed samples that make up the mean seed weight. For comparison, Richardson et al (2015) weight criteria are listed. Seed from two *wyomingensis* and one *vaseyana* sites are similar to published weight criteria while seed at 12 *wyomingensis* and one *tridentata* sites are lighter.

	Field Location	Primary subspecies	n	Mean Weight	99% CI weight range
Meet weight criteria	Beverly Hills	<i>wyomingensis</i>	32	2.319	2.28 mg -3.26 mg
	Five Mile	<i>wyomingensis</i>	40	2.299	2.28 mg -3.26 mg
	Shumway Rd	<i>vaseyana</i>	16	2.837	2.19 mg - 3.44 mg
Fail weight criteria	Beowawe	<i>wyomingensis</i>	20	2.189	2.28 mg -3.26 mg
	Big Wash	<i>wyomingensis</i>	24	2.125	2.28 mg -3.26 mg
	Bonita Rd	<i>wyomingensis</i>	48	2.072	2.28 mg -3.26 mg
	Crowley	<i>wyomingensis</i>	36	1.717	2.28 mg -3.26 mg
	Harper Westfall Rd	<i>wyomingensis</i>	44	1.679	2.28 mg -3.26 mg
	Lytle Blvd	<i>wyomingensis</i>	20	1.8	2.28 mg -3.26 mg
	Milford	<i>wyomingensis</i>	40	2.026	2.28 mg -3.26 mg
	Ryndon	<i>wyomingensis</i>	44	2.212	2.28 mg -3.26 mg
	Silver Zone	<i>wyomingensis</i>	84	1.888	2.28 mg -3.26 mg
	Vines Hill	<i>wyomingensis</i>	52	1.996	2.28 mg -3.26 mg
	West Carlin	<i>wyomingensis</i>	40	2.18	2.28 mg -3.26 mg
	Winnemucca	<i>wyomingensis</i>	96	2.04	2.28 mg -3.26 mg
	Willow Creek Rd	<i>tridentata</i>	40	1.305	1.48 mg - 2.07 mg