

Techniques to determine big sagebrush subspecies in seed lots and why it's important

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Outline

- Does determining big sagebrush subspecies matter
- Seed weight findings
- Analysis of BLMs seed purchases
- Use of a plate spectrophotometer for empirical measurements of UV fluorescence
- Development of a seed testing protocol



Subspecies: does it matter?

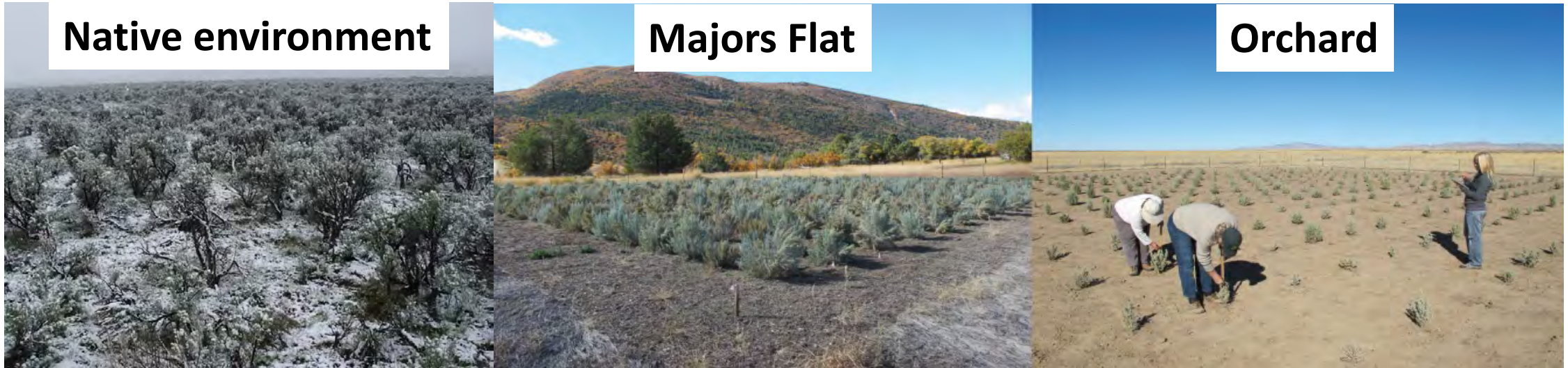


- Research has shown big sagebrush subspecies occupy different habitats:
 - Mtn big sagebrush – higher elevation, mesic
 - Basin big sagebrush – lower elevation, deep soils
 - Wyoming big sagebrush – lower elevation, dry shallow soils
- But overlap can occur at small spatial scales depending on the environmental heterogeneity
- Seed collection perspective

Subspecies: does it matter?

- BLM and Forest Service policy states that the right seed has to be place in the right place
- The mindset has been taxonomic purity is key
 - Big sagebrush is certified to subspecies based on onsite evaluations
 - However, because of scale at which seed is collected and the scale at which restoration is conducted few if any seed collections are purely one subspecies
- Two factors that would better ensure establishment and resiliency of sagebrush restoration:
 1. Post collection evaluation of the composition of subspecies
 2. Matching climate of seed collection to restoration site (seed zones)

Seed weight study: Seeds collected from 3 different environments

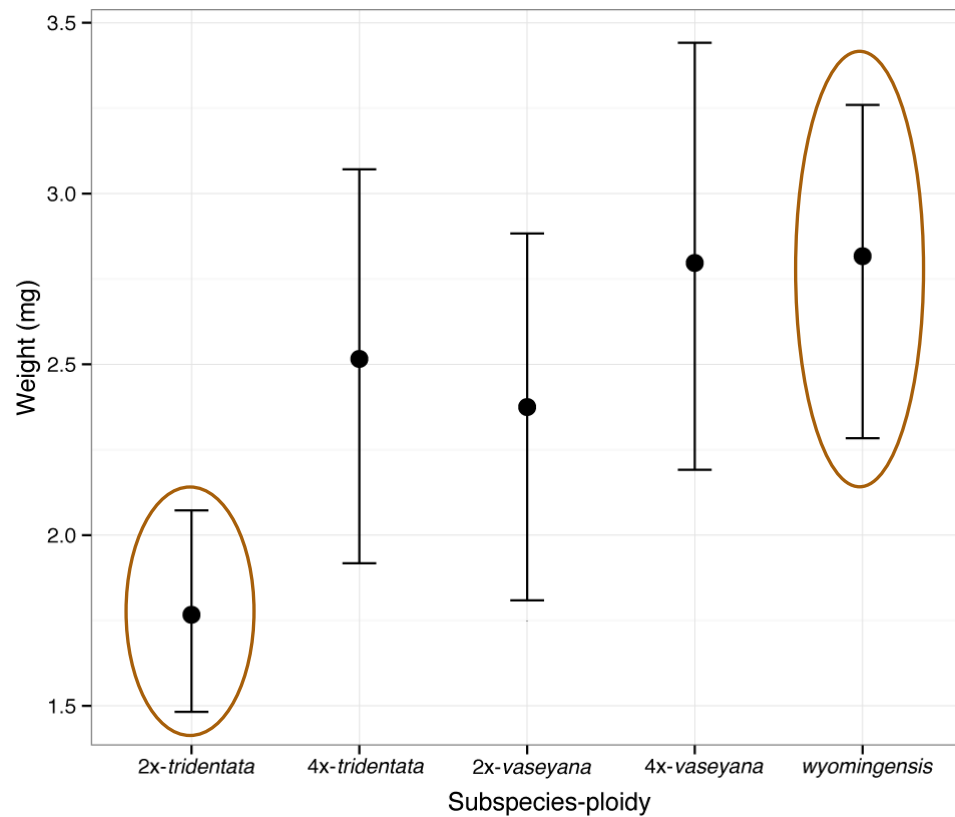


Garden	Elev (m)	MTCM °C	MTWM °C	MAP (in)	Climate
Majors, UT	2105	-4.7	20.8	20.2	Cool/wet
Orchard, ID	974	-2.9	25.0	10.1	Warm/dry

Mixed model results

Random effects	df	Variance	SD	P value
Collection	3	0.0116	0.1080	0.748
Year × Collection	5	0.0247	0.1573	0.004
Population × (Year × Garden)	118	0.0963	0.3104	<0.0001
Family × (Population × (Year × Collection))	443	0.1272	0.3566	<0.0001
Residual		0.0466		
Fixed effects		Estimate	SE	P value
<i>2x-tridentata</i> (intercept)		1.7655	0.1044	0.0032
<i>4x-tridentata</i>		0.7150	0.1096	<0.0001
<i>2x-vaseyana</i>		0.5682	0.0943	<0.0001
<i>4x-vaseyana</i>		1.0412	0.1286	<0.0001
<i>wyomingensis</i>		0.9926	0.0751	<0.0001

Confidence intervals



99% Confidence intervals

- No overlap between 2x-tridentata and wyomingensis
- Expect seed lots with seed weights > 2.2 mg would have a higher proportion of Wyoming big sagebrush

Comparison of subspecies weight parameters to commercial seed lots

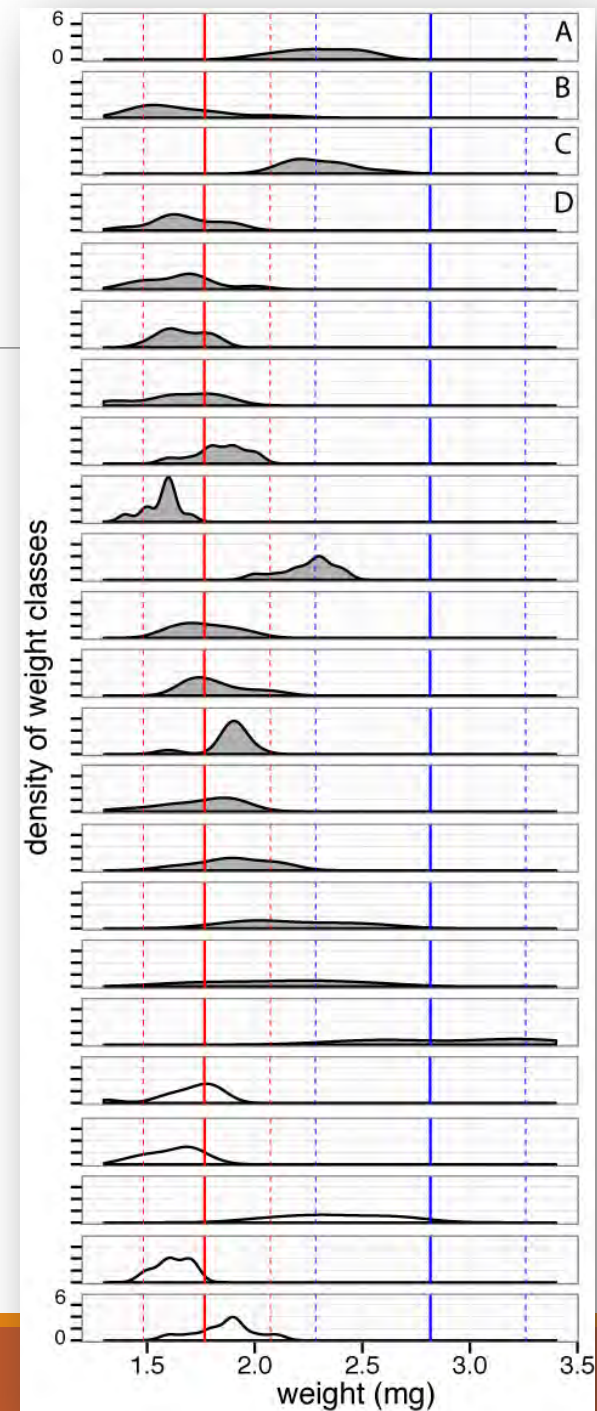
Blue lines = Wyoming big sagebrush

Red lines = basin big sagebrush

Dashed lines = 99% CIs

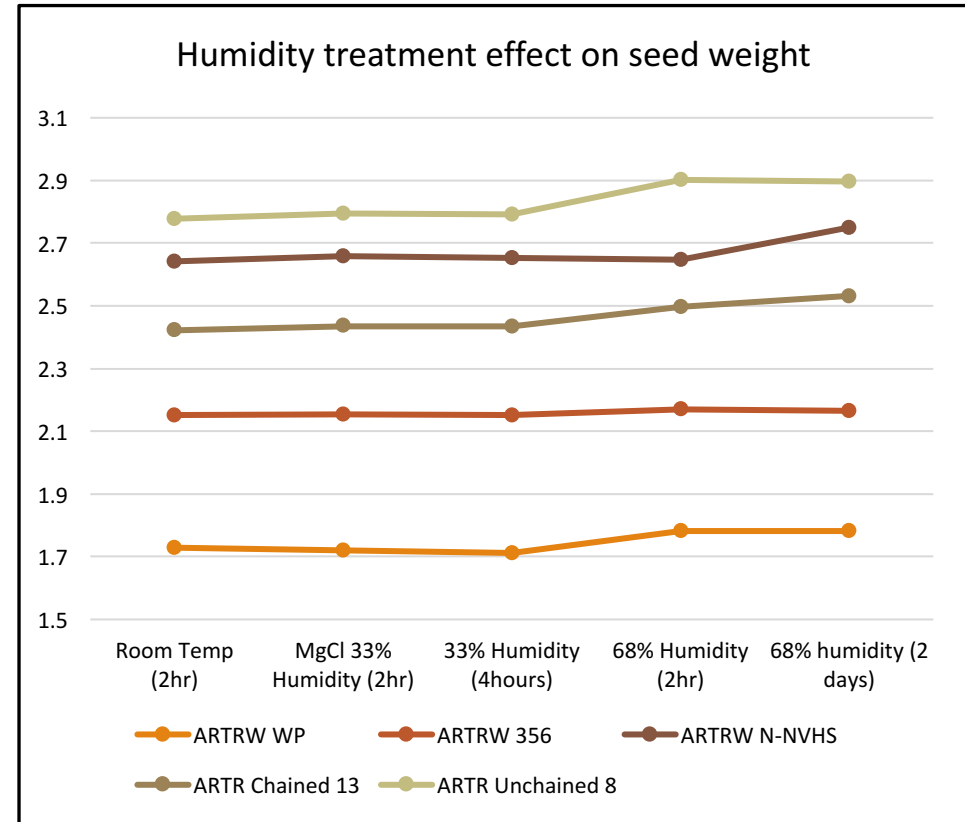
Smoothed distribution based on 10 weighs

Most seed lots labeled as Wyoming big sagebrush contained only a small fraction of this subspecies and were largely composed of Basin big sagebrush



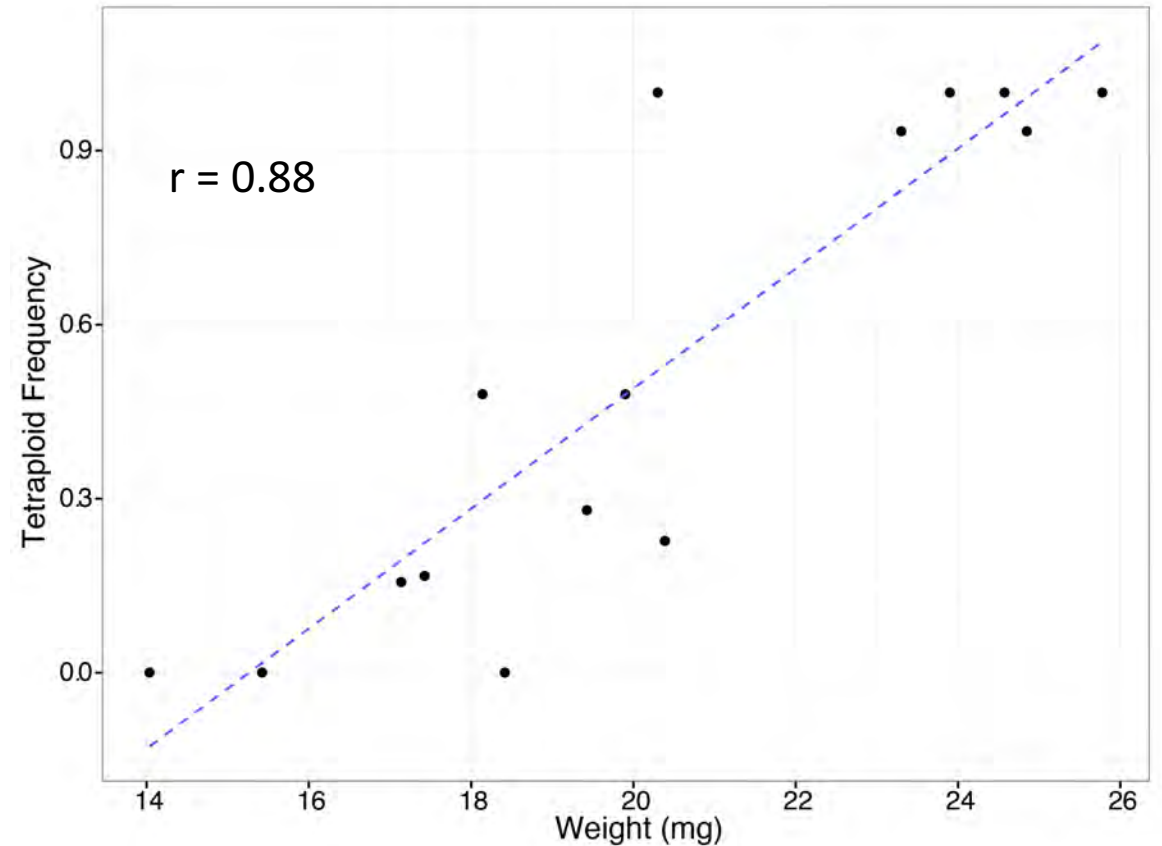
Does humidity effect seed weight?

- Tested seed weight at room humidity (20%) and treatments at 33% and 68%
- Treatment for 2 hrs, 4 hrs and 2 days
- No change between room humidity and 33% and < 0.1 mg between 20% and 68% humidity



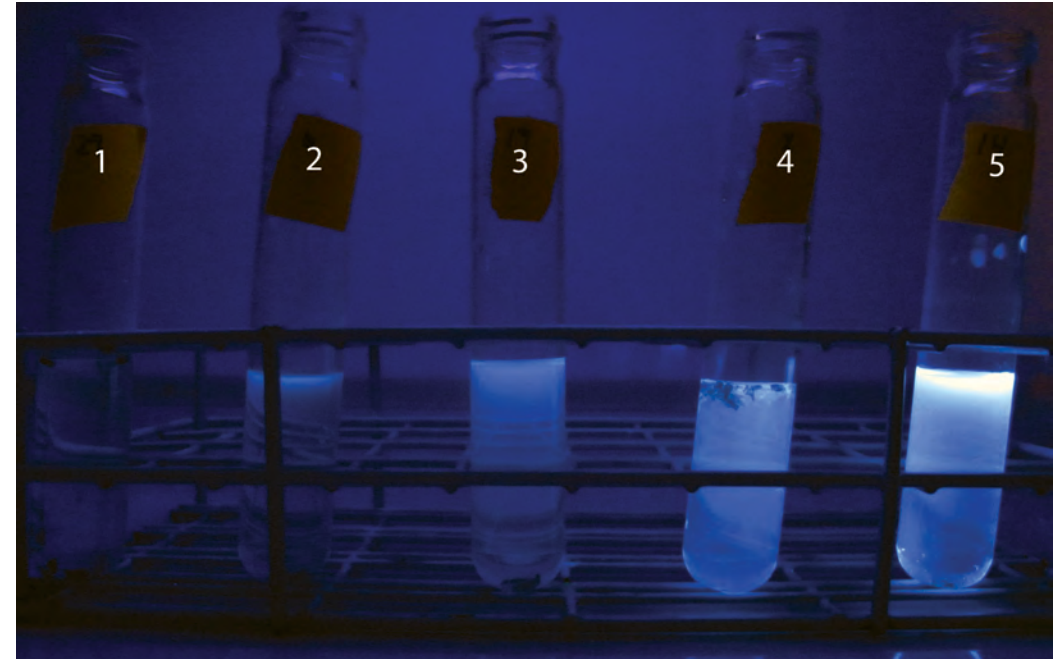
Cytotype frequency vs. seed weight

- Relationship between seed weight and cytotype
- Weight > 2 mg / 10 seeds increases the frequency of *wyomingensis*



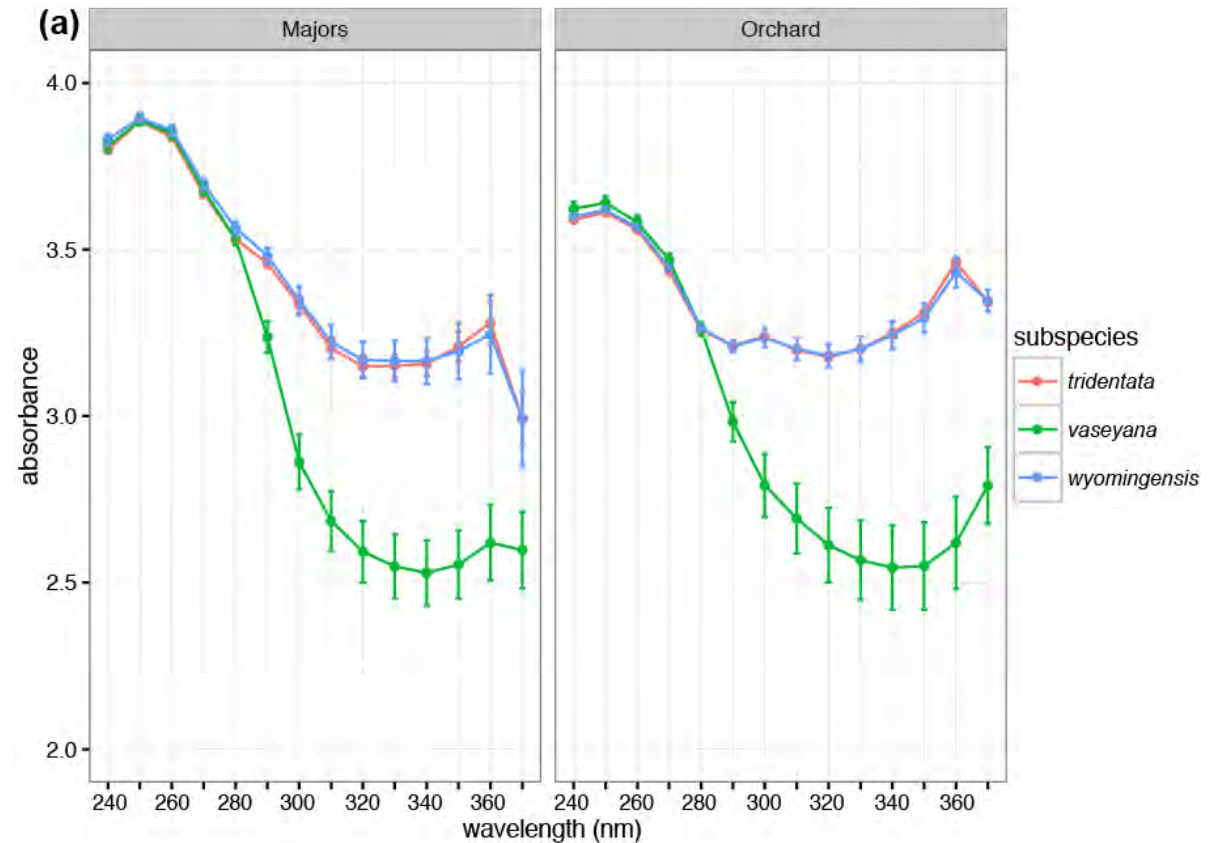
UV fluorescence to determine *ssp vaseyana*

- UV fluorescence by blacklight is diagnostic for mtn big sagebrush
 - Caused by coumarin
 - Fluorescence based on visual rating (1 to 5)
 - Subjective
- An empirical test for UV fluorescence would be a benefit to seed certification



Absorbance curve using a plate spectrophotometer

- Absorbance curves from >600 plants from two common gardens
- Subspecies was previously confirmed by flow cytometry, genetics, etc.
- No overlap between mountain and basin/wyoming spp (95% CIs)
- Wavelength 340 nm showed the greatest difference between subspecies



Mixed model results

Random effects	obs	Variance	SD	P value
Garden	2	0.0012	0.034	0.3
Population × garden	103	0.0142	0.120	2 ^{e-6}
Residual		0.0591	0.2431	
Fixed effects		Estimate	SE	P value
Intercept (tridentata)		3.1873	0.0323	6 ^{e-4}
2x-vaseyana		-0.6822	0.0423	< 2 ^{e-16}
wyomingensis		-0.0246	0.037	0.490

Summary

Environment:

- Effects are small
- These effects do not blur the seed weight differences between Basin and Wyoming big sagebrush
- Or the difference in UV fluorescence between mtn big sagebrush and others

Genetics (subspecies and cytotype differences):

- Seed weight and UV traits are a strongly controlled by genetics
- The most important factor in seed weight appears to be polyploidy and coumarin content for UV

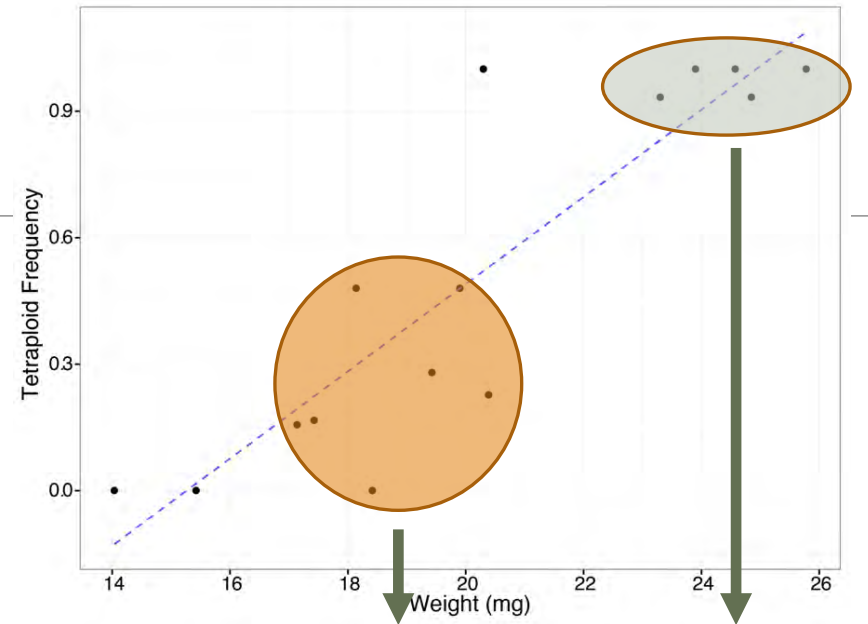
Seed testing

Pure collection of a particular subspecies are generally not a reality. Why try to certify to subspecies?

Determine the composition of Basin and Wyoming plants in seed lots.

Why is this important:

Knowing the proportion of subspecies can help guide where seed lots would be most suitable.



Mixture of Wyoming and Basin



High proportion of Wyoming

Proposed seed testing protocol

Goal: develop a standardized procedure for seed testing that fits within established AOSA seed testing guidelines and assesses the composition of subspecies in each seed lot

The proposed test would include:

1. 8 random samples per seed lot, 100 seeds weighed per sample
2. Chaff from 8 samples used to determine UV fluorescence with spectrophotometer
3. A rating given by the BLM based on Wyoming/basin composition from data supplied by seed labs:
 - Rating A: High Wyoming big sagebrush purity ($\geq 95\%$) to rating D: \geq Low Wyoming seed ($\leq 15\%$)
4. A similar rating system for mtn. big sagebrush vs. basin ssp on UV absorbance score

Final thoughts: Providing a fighting chance

For better establishment and resiliency:

- Identify the subspecies composition of seed lots
- Use big sagebrush seed lots that are most tailored to the site
- Prioritize restoration efforts to areas that will support sagebrush for decades



Collaborators and funding

Collaborators: Nancy Shaw - RMRS, Matthew Germino - USGS,

Technical assistance: Stephanie Carlson, Hector Ortiz, Alicia Boyd, Tanner Tobiasson, Deidre Jaegar, Alexis Malcomb, Matt Fisk, Erin Denney, Jan Gurr, Utah DNR, BLM, and numerous plant seed collectors, Udall laboratory BYU

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This and additional presentations available at <http://nativeseed.info>

