

GENETIC DIVERSITY OF DOWNY BROME IN WHEAT PRODUCTION SYSTEMS

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What is downy brome?

Background

Literature

The Issue

Project Importance

Hypothesis

Methods

Results

Discussion

What is downy brome?

Downy brome is an annual bunchgrass in the Poaceae family that flowers in spring-summer.

**Seeds are dispersed by wind or animals.
AND human shoes and pens.....**





Background

1st invasion: Pennsylvania in 1790 via grain and hay from Eurasia

Reported in Washington 1893

Invades all over US

Yield losses reach 90%!

Why is downy brome a problem?

Downy brome is a highly invasive weed that invades wheat fields and decrease yields

Growers are having difficulty removing it

It is important to be able to maintain a productive wheat system that will support local growers, the community, and an increasing population with diminishing resources.



Literature

Novak et al. studied 60 populations,

-average heterozygosity = 0.012

-Very little outcrossing

Merrill et al. looked at 1920 individuals across 96 locations around US

-14 biotypes = 79% total individuals

Project Importance

Studies of downy brome have been conducted in natural, rangeland settings

This study focuses on the adaptation of downy brome in wheat production systems, which has never been studied before.

Goals and Objectives

- *Study the diversity of downy brome in wheat field production systems

- *Characterize phenotypes associated with downy brome: seed dormancy, after-ripening, germination.

Methods

Phase 1: Collecting in-field samples

Phase 2: Seed germination

Phase 3: DNA extraction

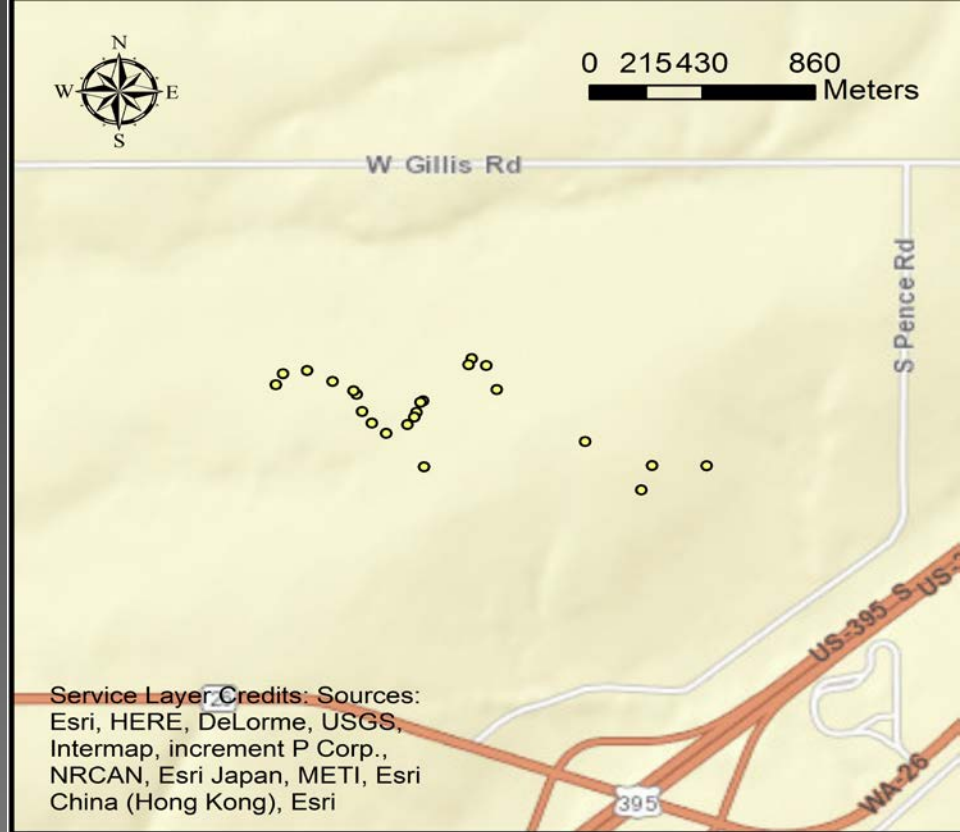
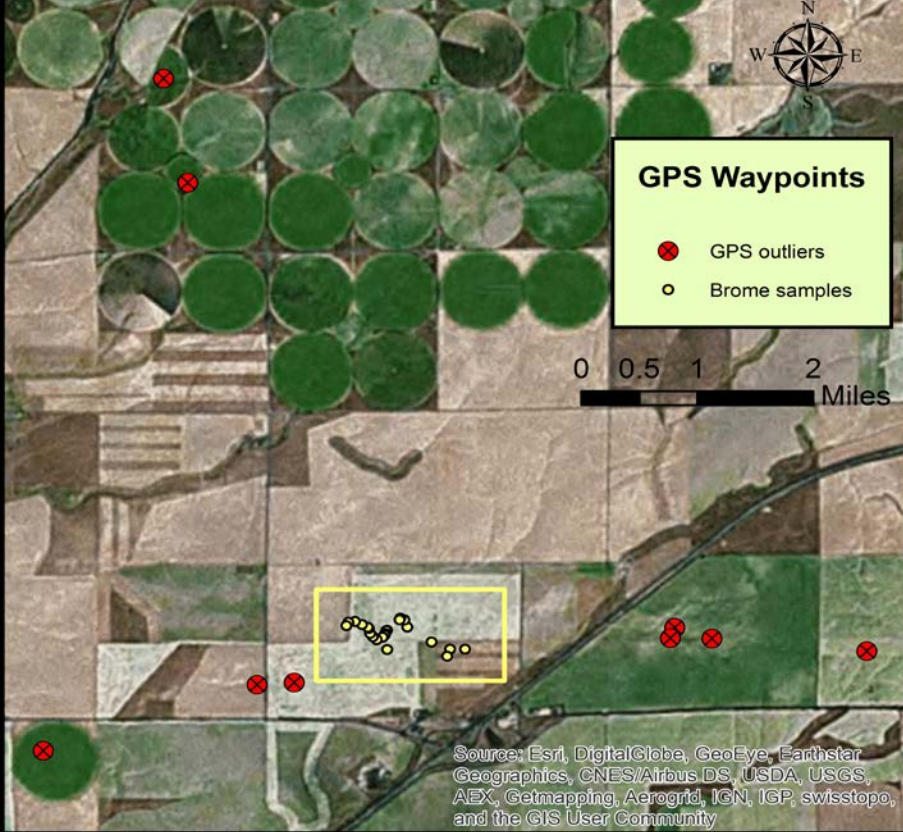
Phase 4: Primer Design and PCR

Phase 5: Gel electrophoresis

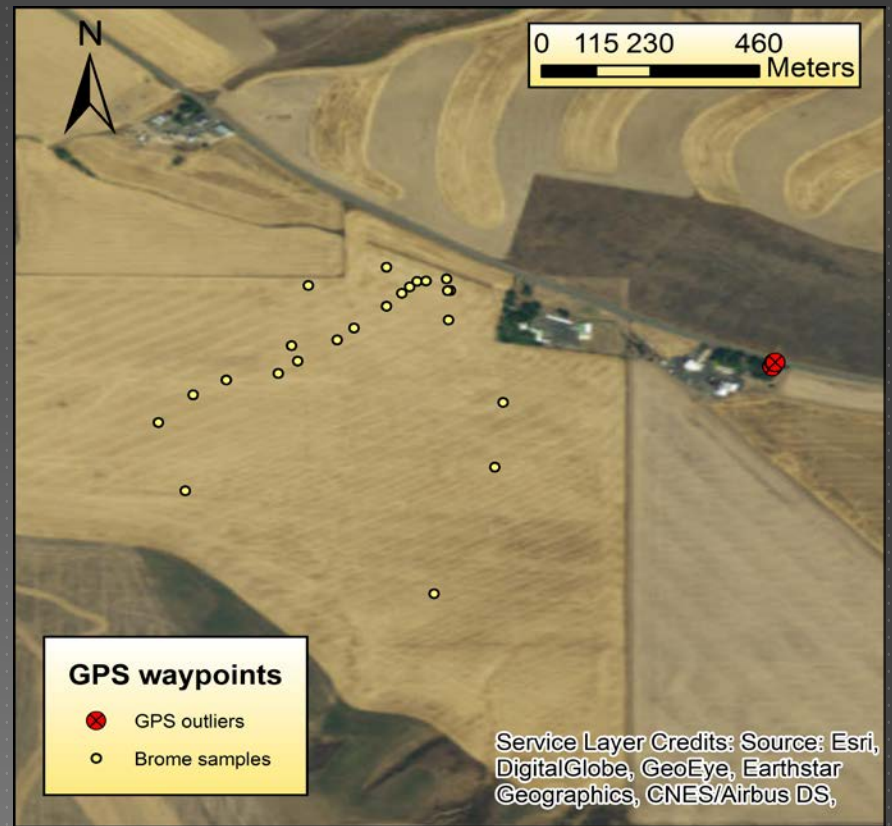
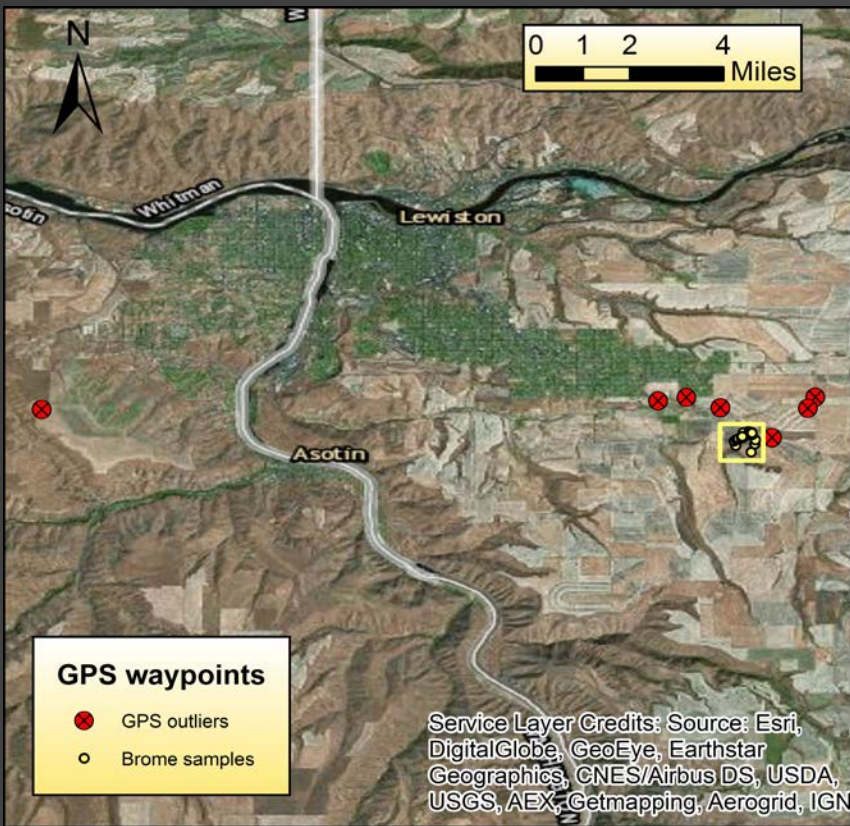
Phase I: Field Sampling

Visited 8 different sites (McGregor wheat fields)

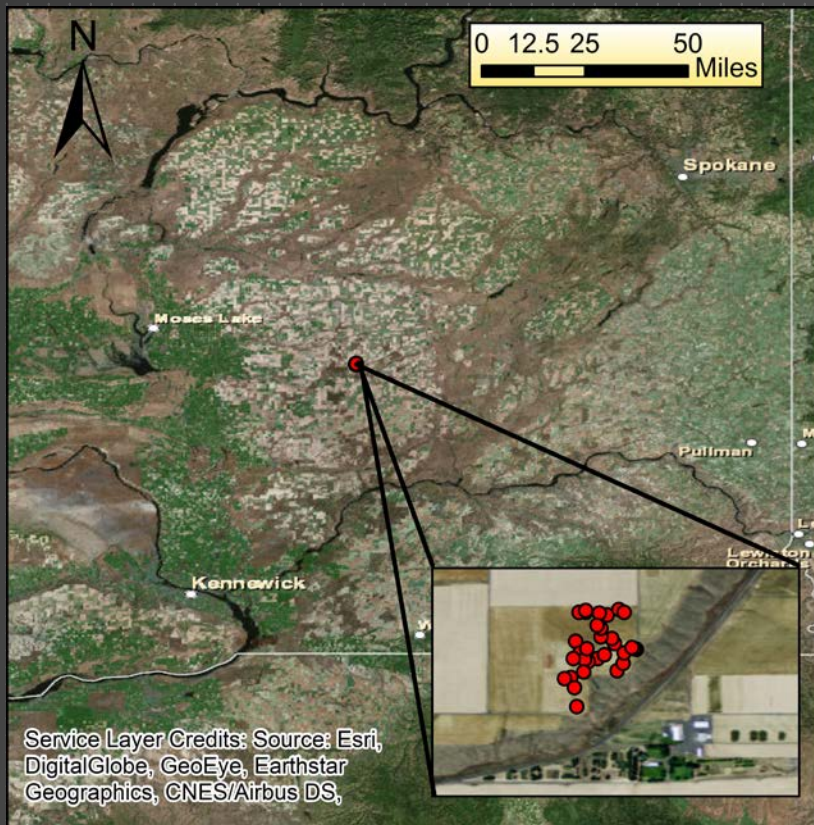
-6 in WA, 2 in ID



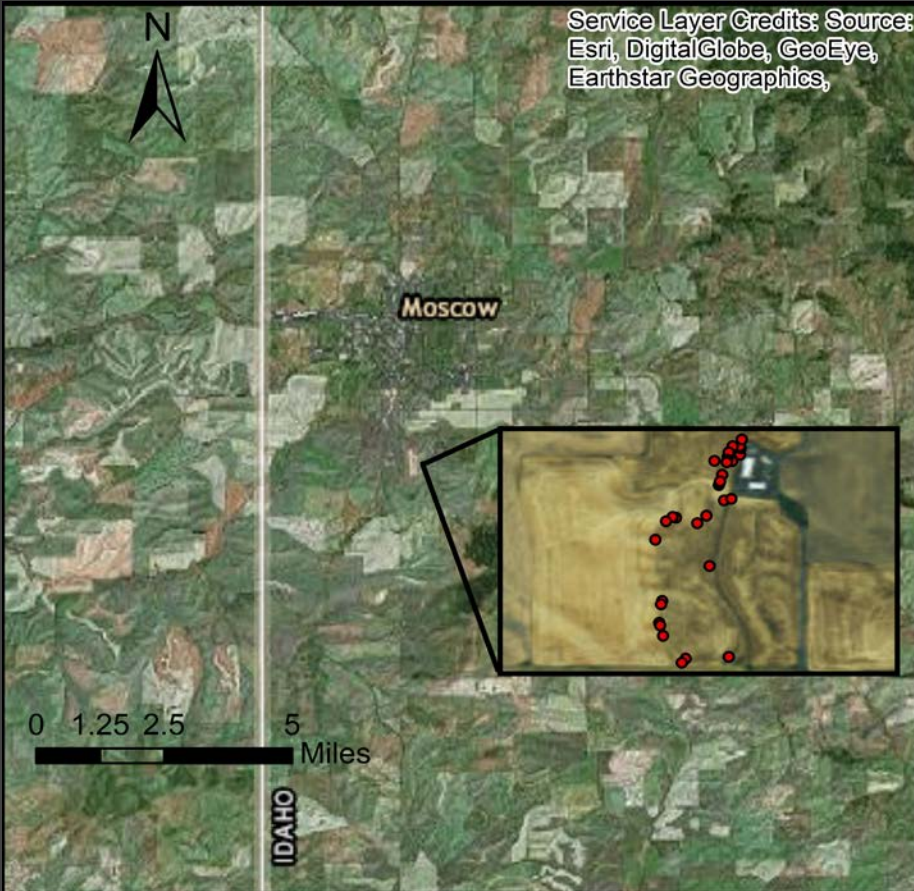
Dusty, WA



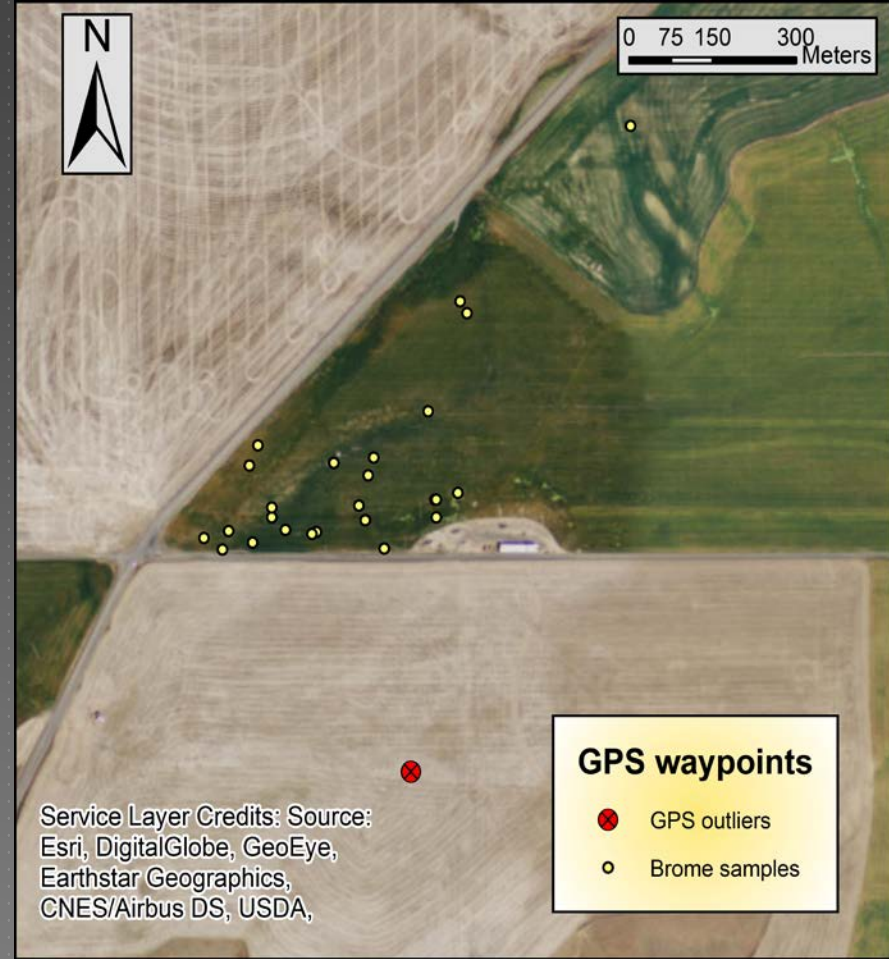
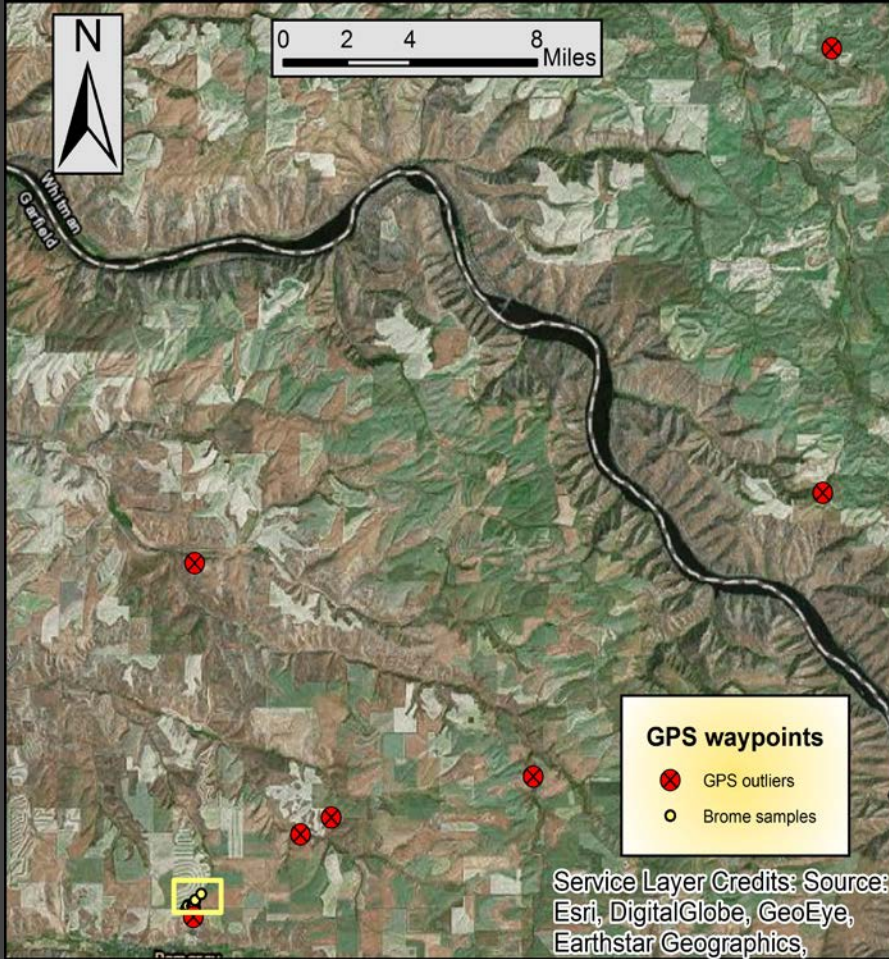
Lewiston, ID



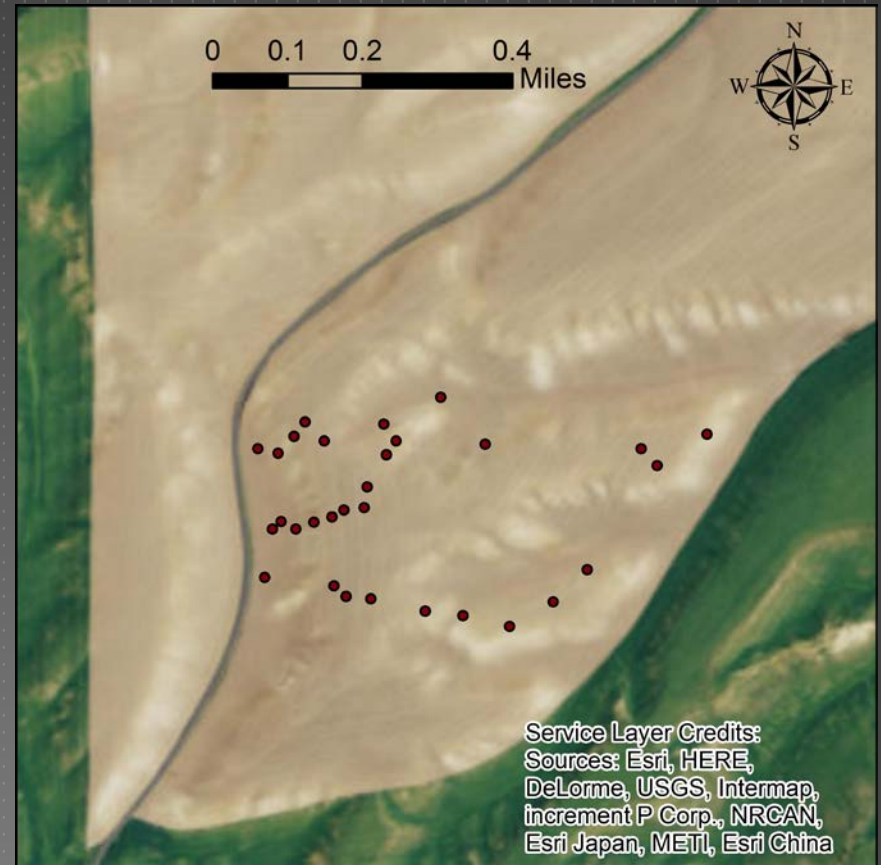
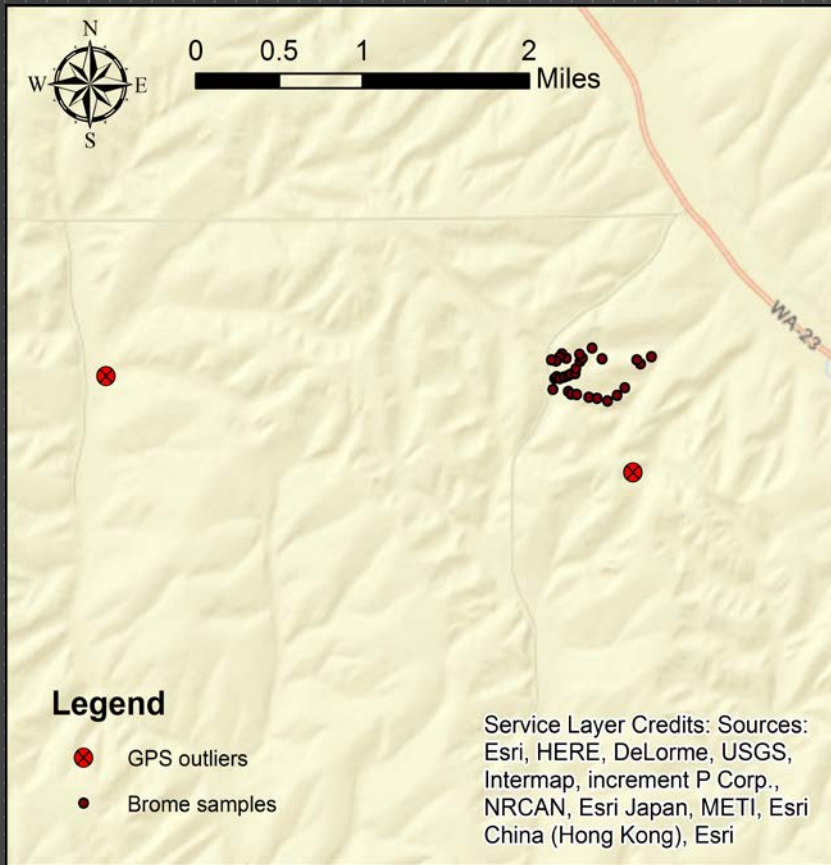
Lind, WA



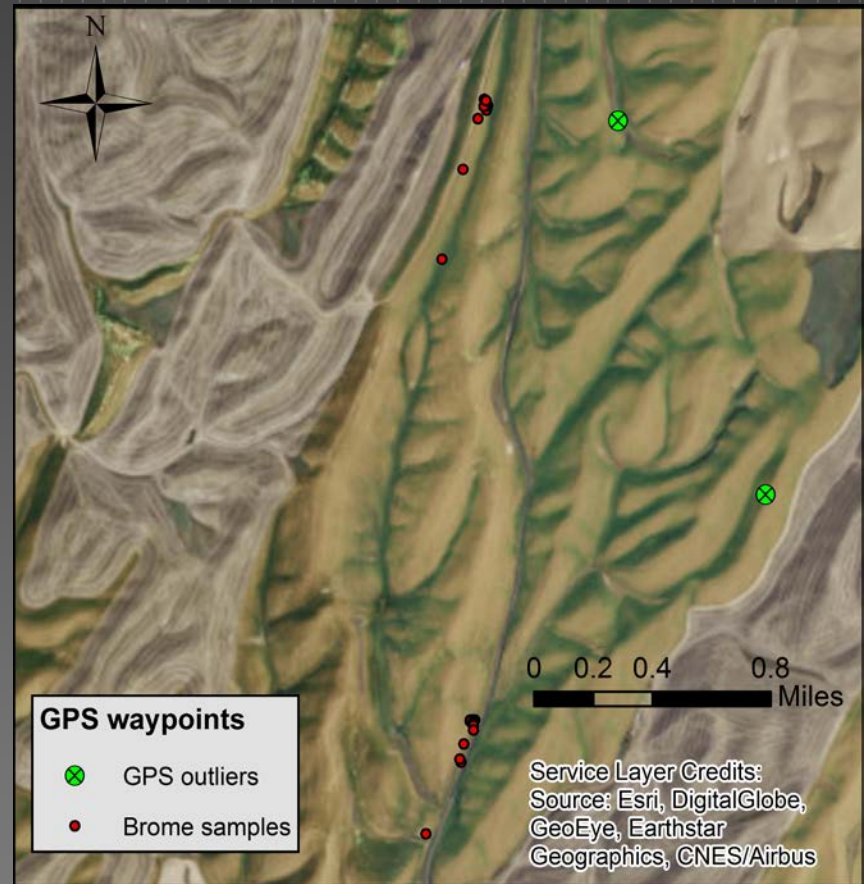
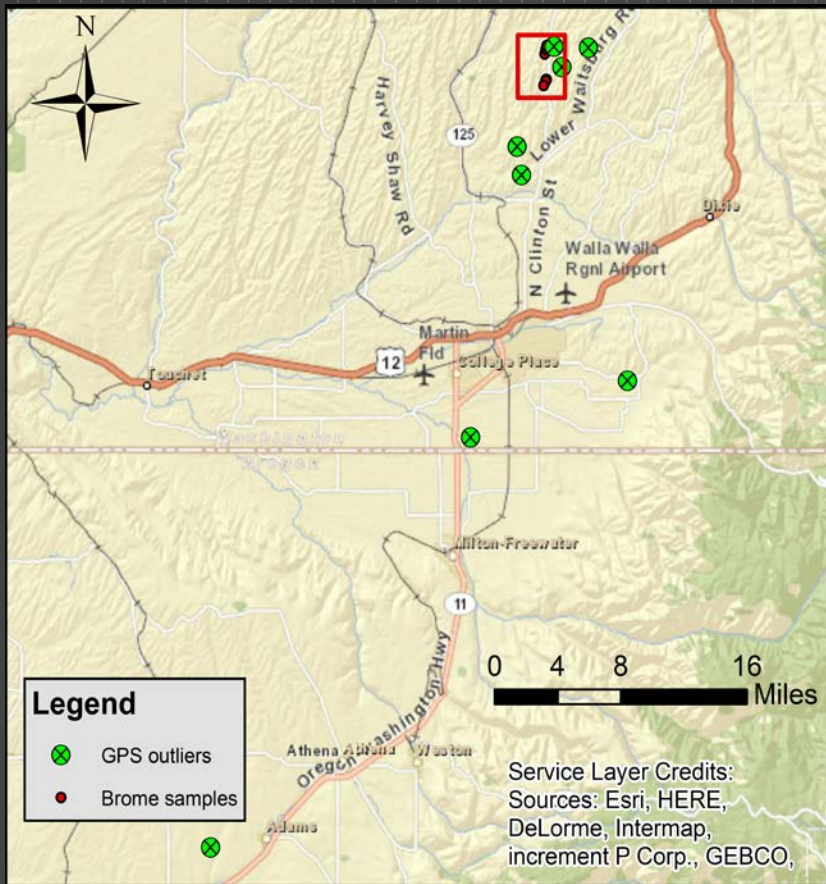
Moscow, ID



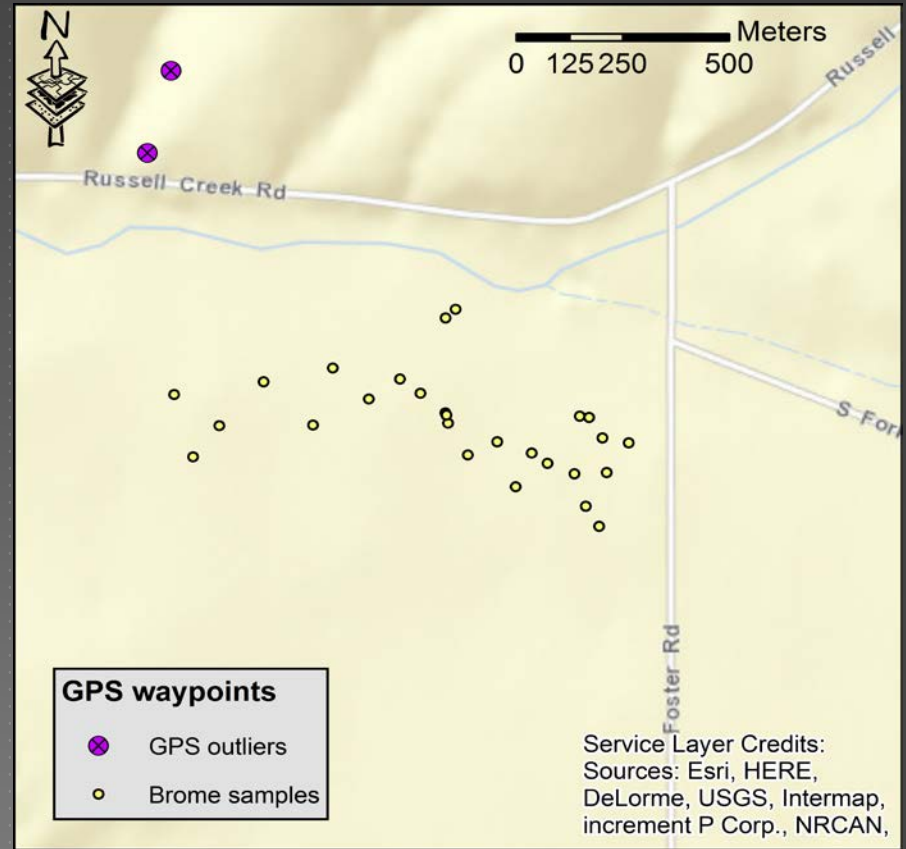
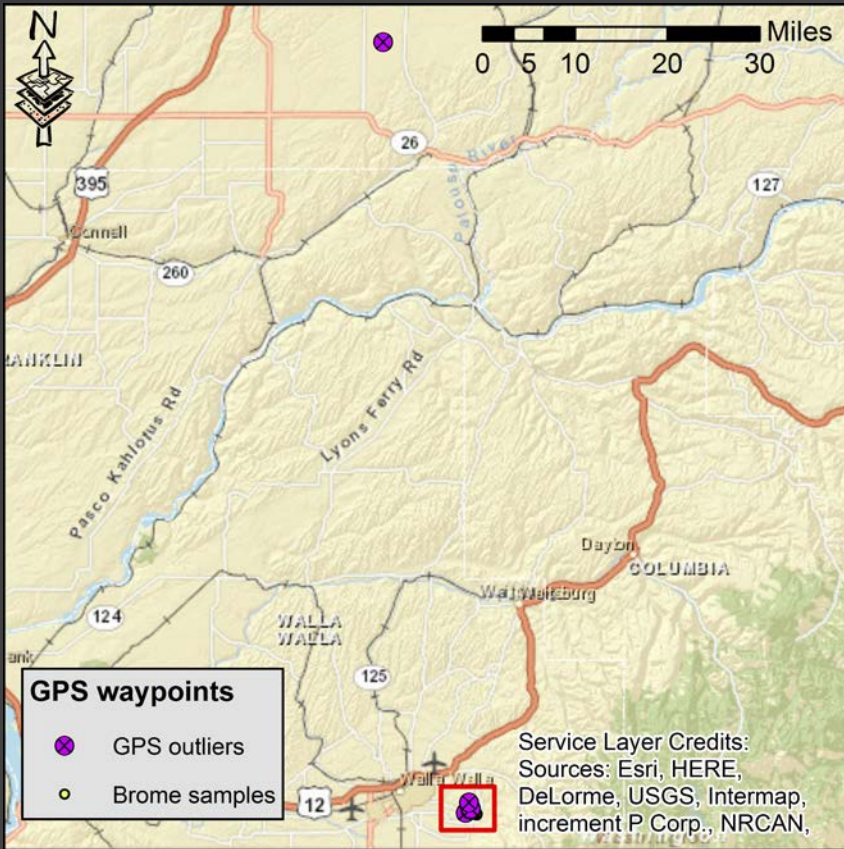
Pomeroy, WA



Sprague, WA



Waitsberg, WA



Walla Walla, WA



Samples collected via “pseudo” random sampling

32 plants collected from each field

Plants were collected in bags and hand threshed in lab



Phase 2: Germination

Preliminary germination

Prelim Trial 1:

- 4 treatments: GA+GC, -GA+GC, GA+Vern, -GA+vern (25mL, 10mM GA3)
- caryopses germinated in plating bags
- vern treatment= 4 days → GC
- miracle grow
- 28 seeds/treatment

Prelim. positive trial results:

+GA -V= 39%

+GA +V= 7%

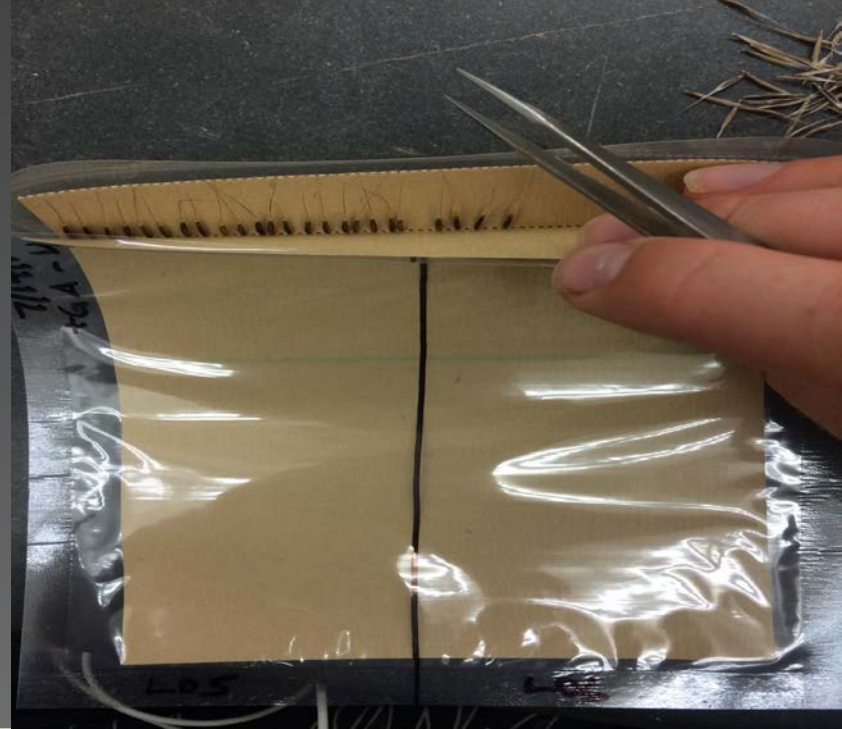
-GA+GC= 0

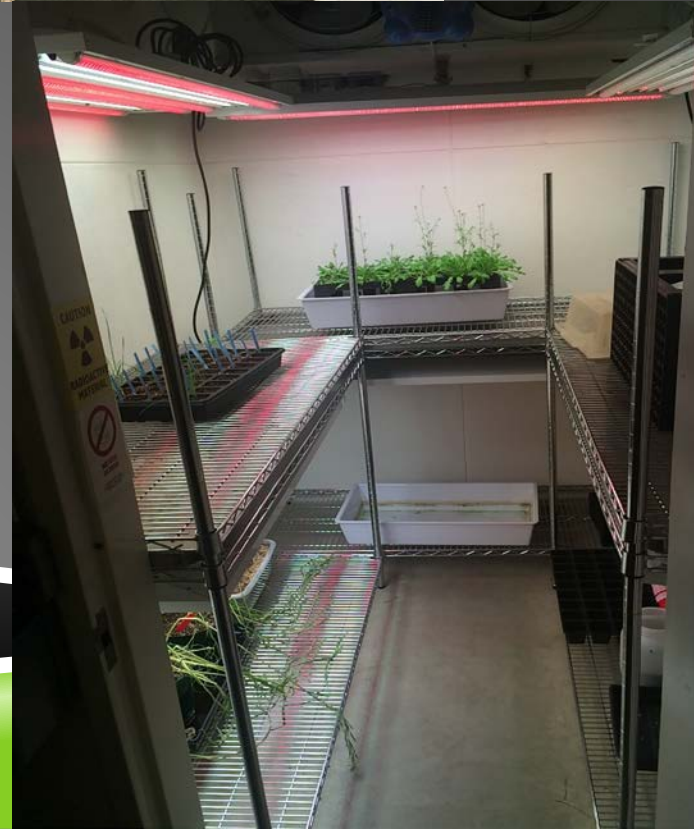
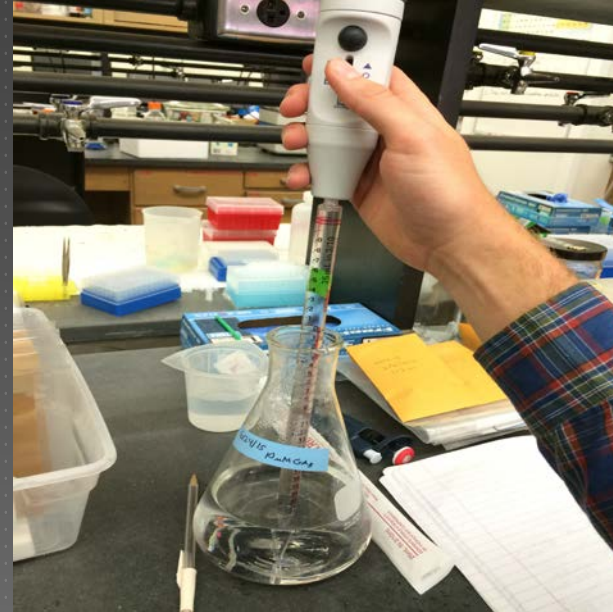
-GA+V= 0

Preliminary trial 2:

- oven treatment at 30°C 1-7 days

- added 20 μM GA3 and put in GC after oven





Preliminary trial 2 results:

1 Day: 31%

2 Days: 19%

3 Days: 30%

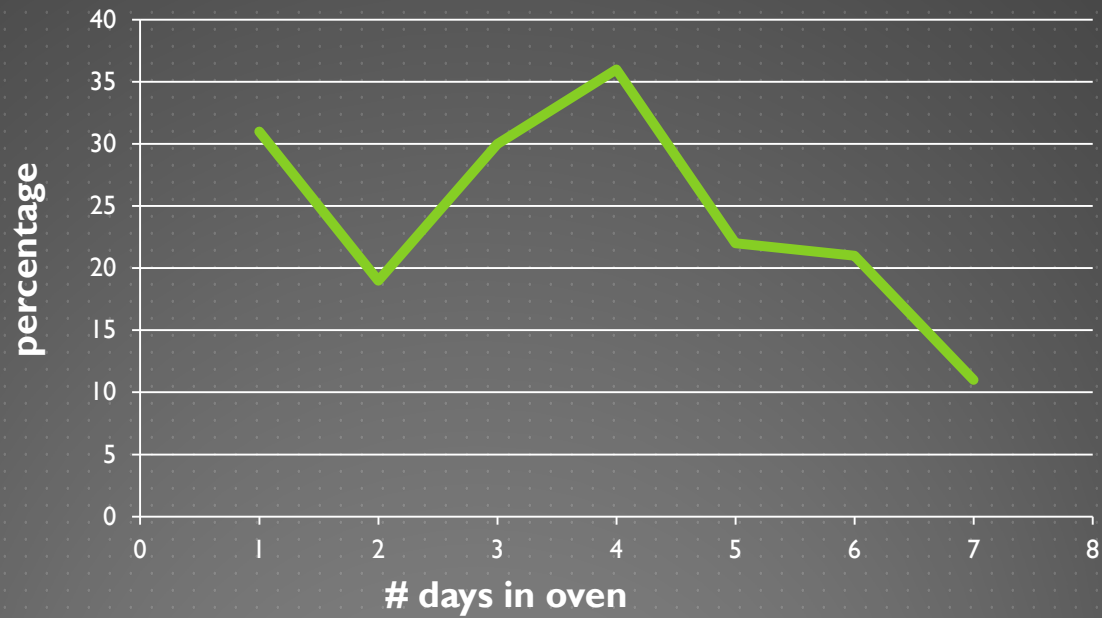
4 Days: 36%

5 Days: 22%

6 Days: 21%

7 Days: 11%

Preliminary Germination Results



FINAL GERMINATION

Treatment : 10 μ M GA3 + GC

4 sites:

Dusty, WA

Lind, WA

Sprague, WA

Pomeroy, WA

32 plants/site, 20 seeds/plant= 640 seeds/site

Final Germination

Dusty, WA=8%

Sprague, WA=3%

Lind, WA= 9%

Pomeroy, WA= 22.5%

Phase 3: DNA extraction

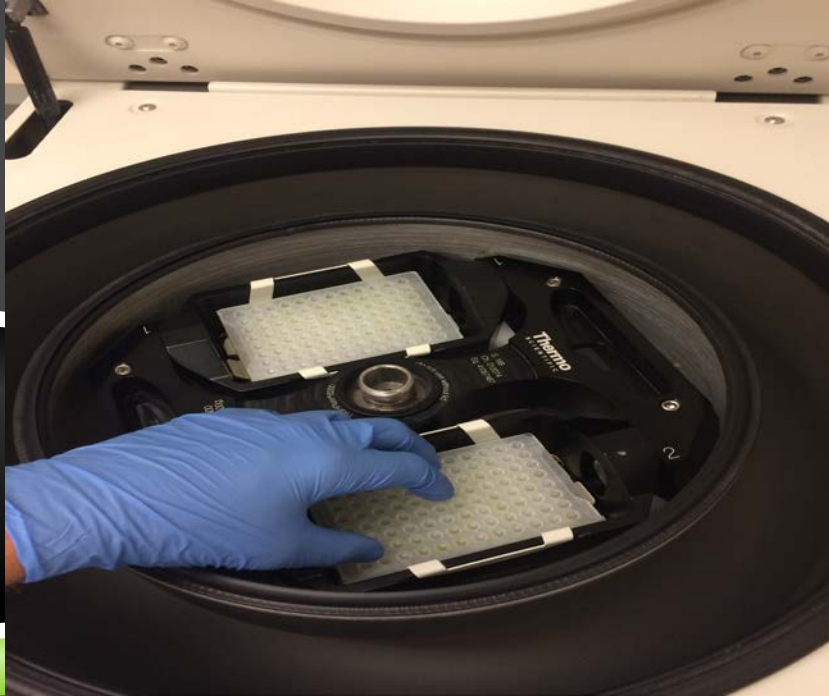
2 methods for extraction:

1) CTAB method

- liquid N
- buffer, isochloroform, isopropanol, ethanol
- separating carbohydrates, proteins, and nucleic acids

2) High through-put-Biosprint 96 robot

- plating compounds into wells to be loaded into BioSprint robot
- 5 blocks: tween, ethanol, lysate, h20, buffer, isopropanol, magnetic beads

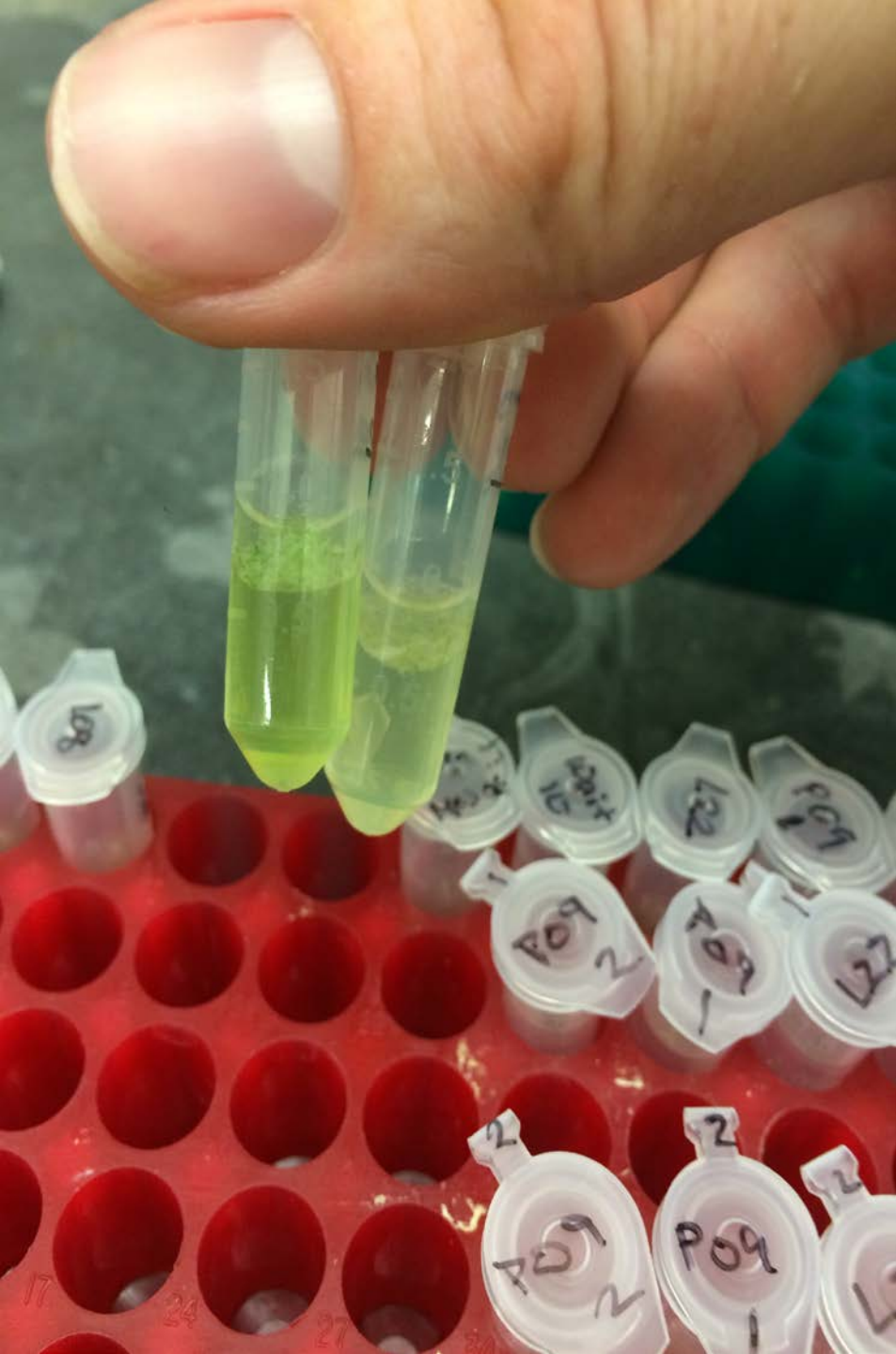


shake the entire rack in
Ensure that the
inverts the rack of

Note: For elution of DNA, use distilled water that does not contain
Tween 20. *add detergent removed from beads from beads*

1. Transfer 200 μ l cleared plant lysate into each well of an S-Block.
2. Transfer 200 μ l isopropanol to each sample in the S-Block.
3. Add 200 μ l isopropanol to each sample in the S-Block.





Phase 4: Primer Design and PCR

Primer: short strand of nucleic acid sequences used as a starting point for DNA synthesis

PCR-polymerase chain reaction, used to amplify a DNA sequence by creating many copies of a sequence

Background for Primer Design

Nevin Lawrence collected 96 plants from different GPS locations

Distinguished 6 unique populations from all samples

8 most informative 100bp sequences were found, each associated with a SNP

SNP (single nucleotide polymorphism)- represents a difference in a single nucleotide

e.g. ATTCG
ATACG

Primers were designed from the 8 sequences

I3962_Population 5

Forward Primer



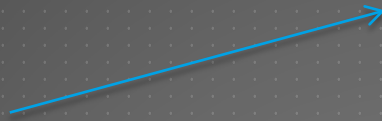
ATCTGTAGAATCACGGAGGAC

...ATCTGTAGAATCACGGAGGT **C**TCCTGTGCGCAGAGTTG

...TAGACATCTTAGTGCCTCCAGAGGACAGCGTCTCAAC

CAACTCTGCGACAGGA

Reverse Primer



PCR

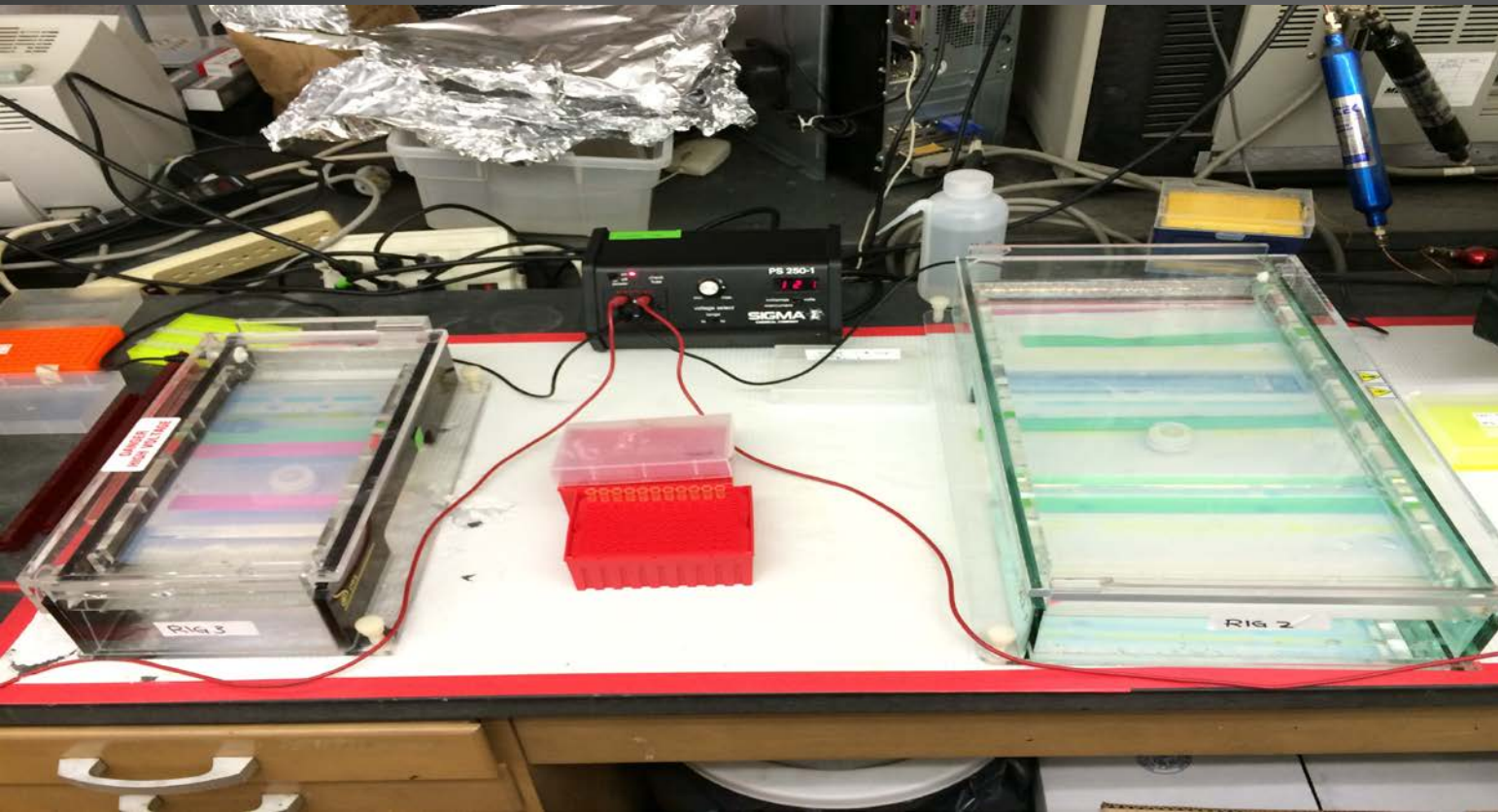


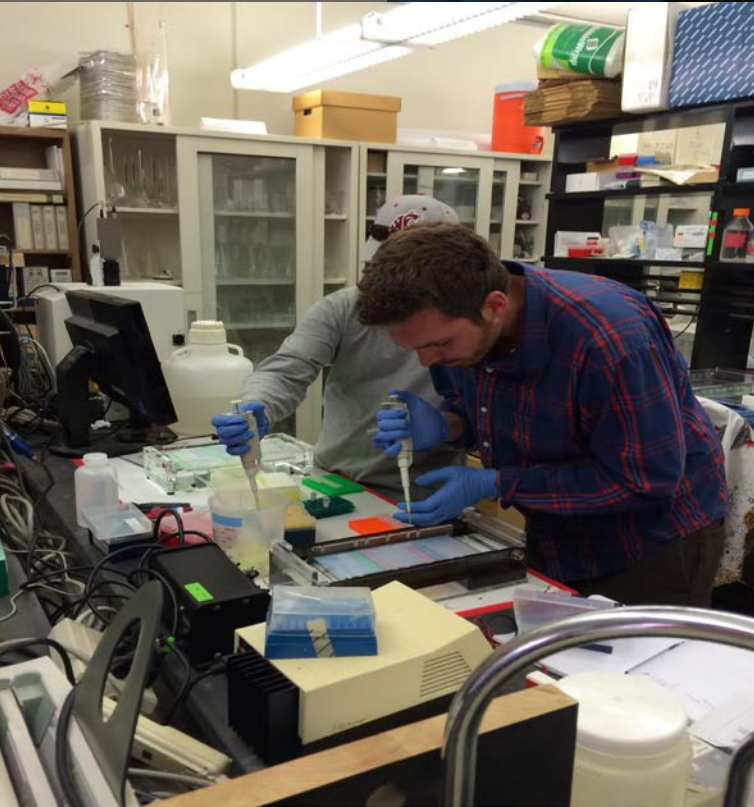
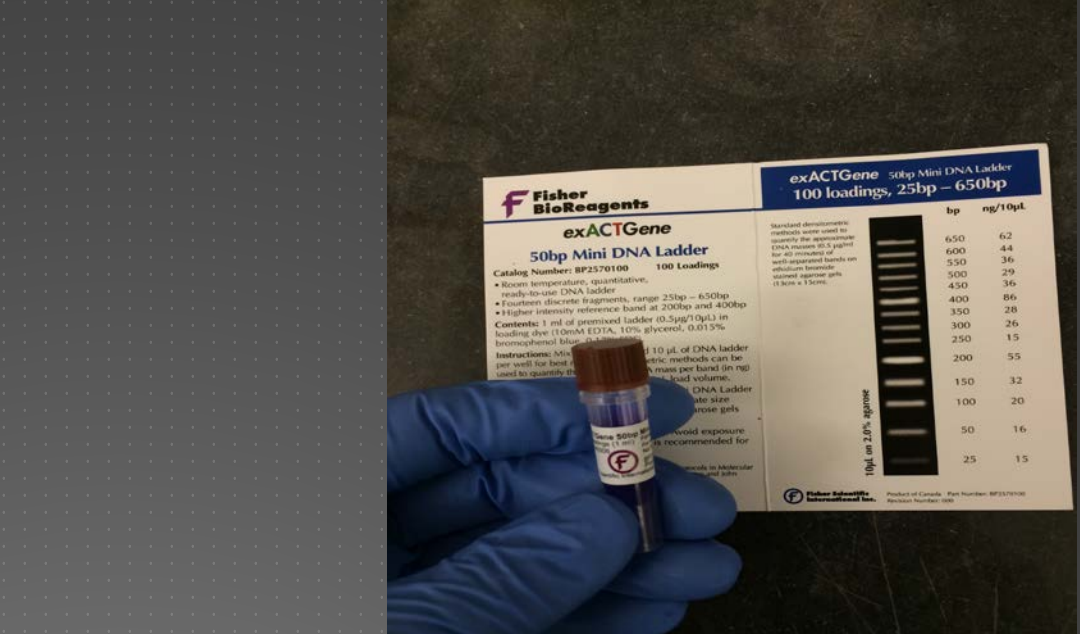
PI P2 P3 **P4** P5



GEL ELECTROPHORESIS

A method for amplifying and separating DNA fragments of different lengths from one another to determine the size of the DNA

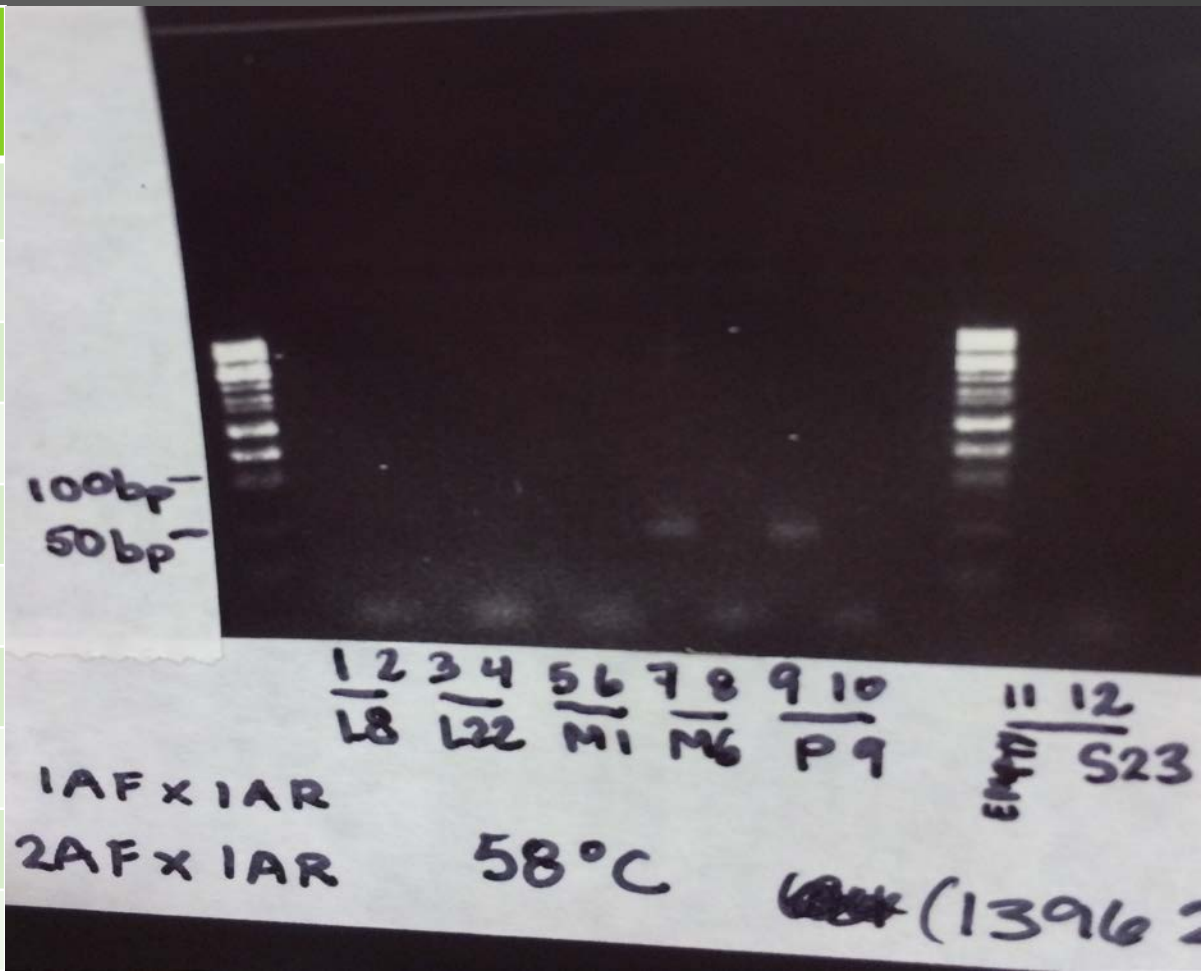




RESULTS

One of the “wild-type” primers was specific to an individual in Pomeroy and Moscow but not for the other individuals including other individuals from Moscow and Pomeroy.

Sampled Site	Primer specificity
Pomeroy #9	+
Pomeroy #29	-
Sprague #13	-
Sprague #23	-
Waitsberg #10	-
Lind #8	-
Lind #22	-
Moscow #1	-
Moscow #6	+



DISCUSSION

Results indicate there may be multiple genetically diverse populations within a single field.

It is important for wheat growers to know how much diversity is in-field in order to better manage downy brome

STAKEHOLDERS

Wheat growers, farmers

Chemical/herbicide Industry

Researchers

Consumers

General public

Sources

Novak S, Mack R, Soltis D (1991) Genetic variation in *Bromus tectorum* (Poaceae): population differentiation in its North American range. *Am J Bot* 78:1150–1161

Merrill KR, Meyer SE, Coleman CE (2012) Population genetic analysis of *Bromus tectorum* (Poaceae) indicates recent range expansion may be facilitated by specialist genotypes. *Am J Bot* 99:529–37

Wikipedia-general definitions

Thank you

WSU Weed Science Lab

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Ian Burke

Nevin Lawrence

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Kyle CB Nicholas Race



QUESTIONS?