

## IFAFS Cheatgrass Control & Range Restoration Project

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### Seed Bank Sampling Protocol

#### FIELD SAMPLING

To evaluate the pre-existing seed bank, we will sample the Experiment 2 area (Figs 1-3) after the last herbicide application and before the fall seeding.

We will estimate the number of seeds, by species, present on the soil surface and in the top 5 cm of the soil profile. Therefore, each sample will be separated in two portions: (1) **litter and surface** seeds, and (2) **0-5cm** depth.

At each site, 8 composite samples per block will be taken ( $n=48$ ); these will be located immediately adjacent to the study plots to avoid disturbance. Study plots will be numbered consecutively, and eight of these will be randomly selected to provide the locations for seed bank sampling. Each composite sample will consist of 4 subsamples (cores) taken 5 cm into the buffer zone from the midpoint on the right side of the selected study plot (Fig. 4).

At each sample location, a 0.1 m<sup>2</sup> quadrat (31.6 x 31.6 cm) will be placed on the ground. A small core (2" inside diameter steel pipe) will be located at each of the four corners of the quadrat. First, the surface litter and seeds within each of the four cores will be collected, placed in the same bag and mixed. Then the soil (0-5 cm depth) from all four cores will be collected, placed in the same bag and mixed. Samples are to be placed in sealed plastic bags, and carefully labeled with date, site, block, plot and depth.

Number of samples per state: 2 sites x 6 blocks x 8 sampling points x 2 depths = 192

#### Note:

1. Carefully avoid collecting aboveground standing vegetation. Also, we will not sample directly on perennial bunchgrasses therefore if you encounter bunchgrasses at the sampling point move 10cm in either direction.
2. If the samples are not stored immediately, open the bags and let them air-dry until processing.

#### SEED BANK EVALUATION

The seed bank will be evaluated by direct germination in a glasshouse after wet-cold stratification. At the lab, samples will be moistened approximate to field capacity (the point at which the soils are thoroughly moist without any standing or free water) and placed in cold storage (~ 1-2 °C) for 60 days. The litter samples will be mixed with ~ 300 g of sterilized sand, and then moistened.

After stratification, each composite sample will be spread over moistened sterilized sand in trays, and watered to stimulate germination under natural light. Soil will be kept moist throughout the entire period. As seedlings became identifiable they will be identified, counted and carefully removed. Unidentifiable seedlings will be transplanted to pots and grown until they can be identified. When emergence is no longer observed, samples will be allowed to dry for 30 days and then rewetted to test for further emergence.

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Experiment 2: Competitive Interactions

2.a. Core experiment (low precipitation study areas in NV and OR, high precipitation study area in UT)

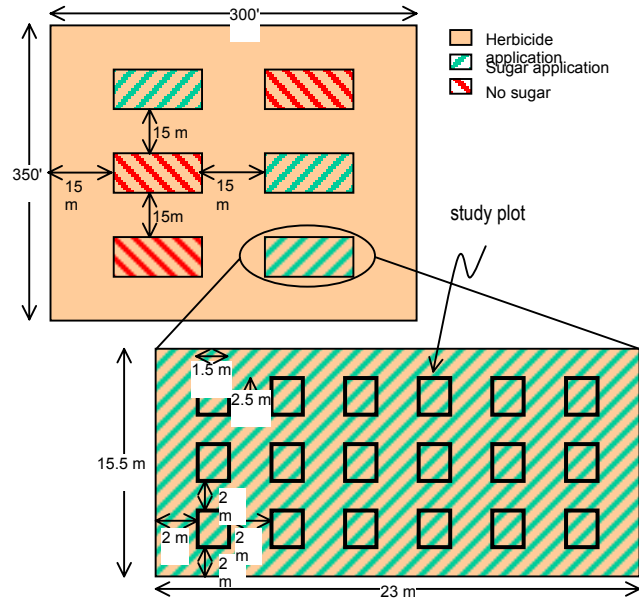
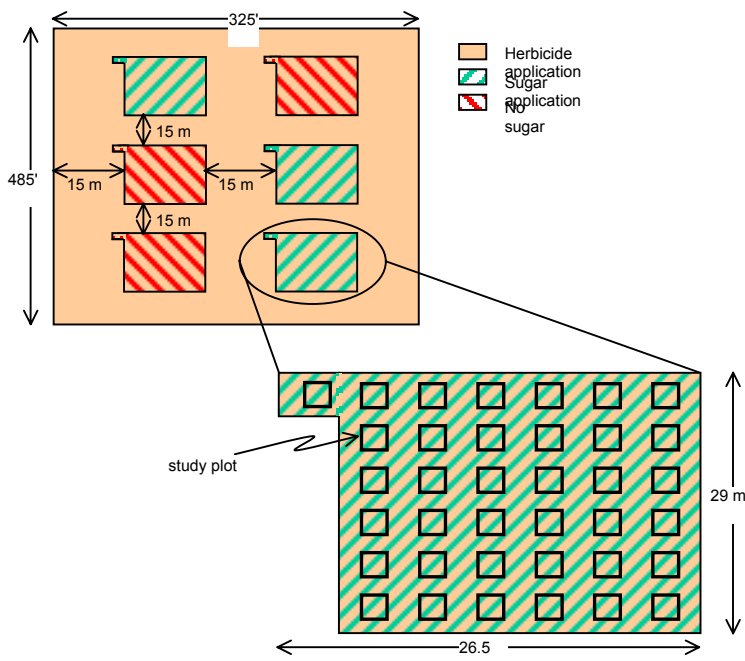


Fig. 1. Plot configuration for 1st year's competitive interactions studies.



2.b. Effects of seed densities (high precipitation study area in Nevada)

Fig. 2. Plot configuration for 1st year's competitive interactions that also investigates seeding density in Experiment 2

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## 2.c. Effects of secondary weeds (both study areas in Idaho, high precipitation study area in Oregon and low precipitation study area in UT)

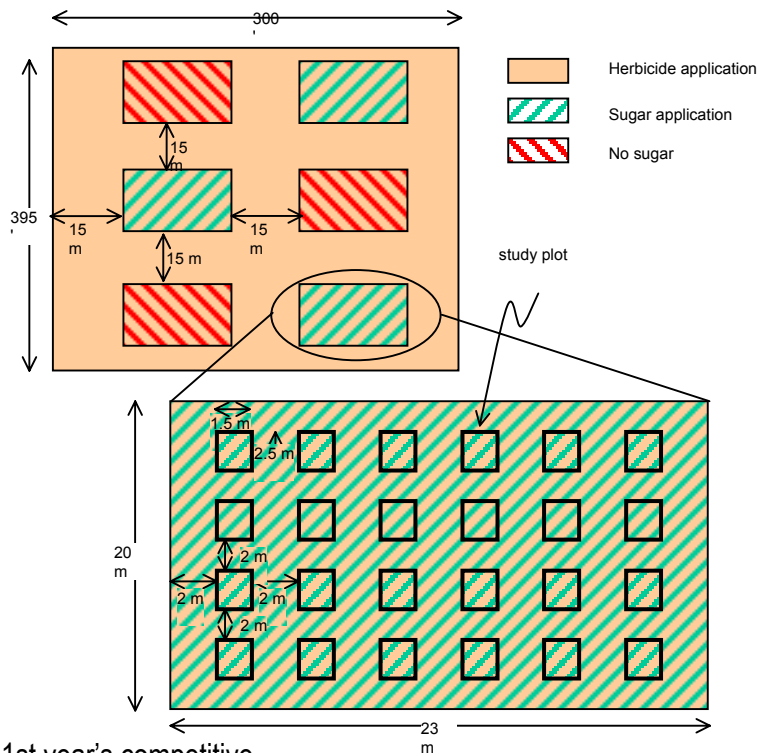
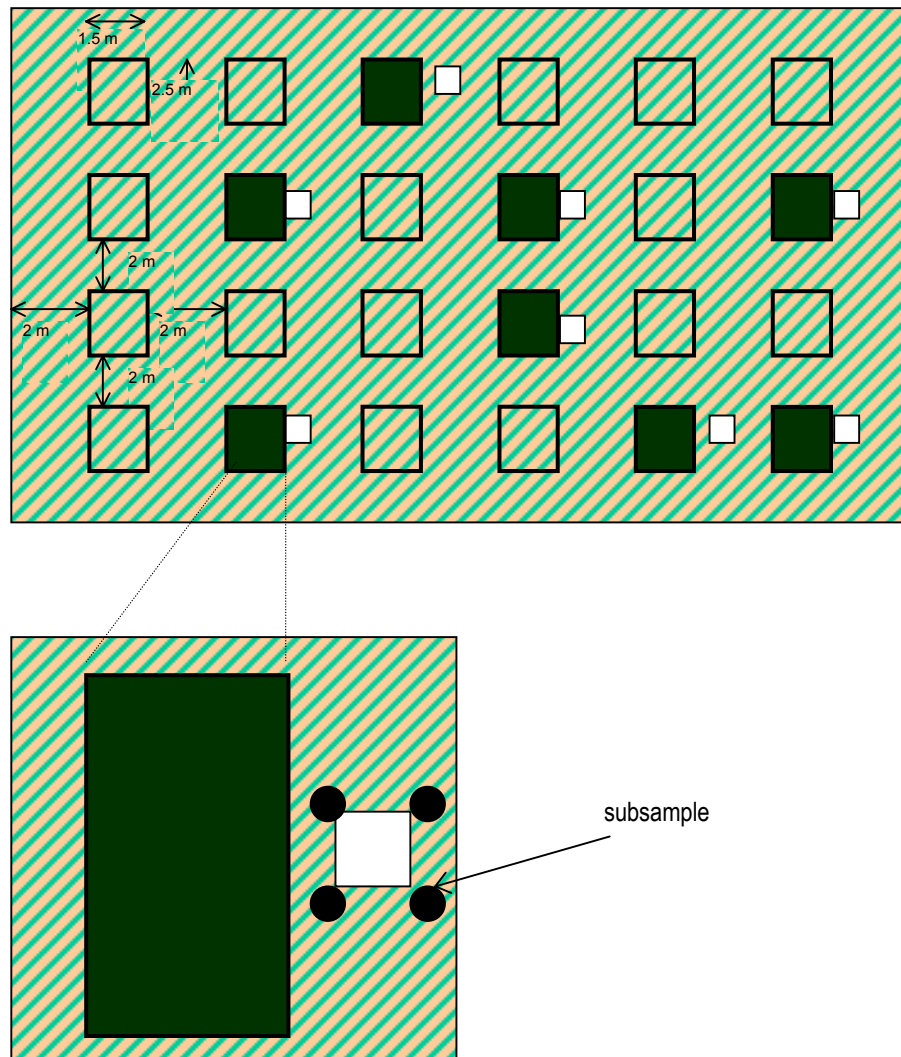


Fig. 3. Plot configuration for 1st year's competitive interactions studies that also investigates secondary weeds in Experiment 2.

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Figure 4. Location of seed bank sample points in relation to study plots showing randomly selected study plots = ■ ; seed bank sampling locations= □ and subsample sampling locations = ● .



### 1. REFERENCES

- Bigwood D.W. and D.W. Inouye. 1988. Spatial pattern-analysis of seed banks: an improved method and optimized sampling. *Ecology* 69: 497-507.
- Chambers J.C. 1993. Seed and vegetation dynamics in an alpine herb field: effects of disturbance type. *Can. J. Bot.* 71: 471-485.
- Humphrey L.D. and E.W. Schupp. 2001. Seed banks of *Bromus tectorum*-dominated communities in the Great Basin. *Western North American Naturalist* 61: 85-92.