Experiment 1: Native Plant Screening Trials (Revised March 4, 2004)

This experiment evaluates the outcome of drill seeding 25 accessions into plots containing cheatgrass or into plots where cheatgrass has been controlled with herbicide.

**Pretreatment Measurements**

Efforts are to characterize the vegetation environment into which seeds are placed.

**Variables**

1. **Pre-seeding vegetation cover** – In order to characterize the cover of plants, especially cheatgrass and other weeds, before weeds are removed and the seeding occurs, run two 25-m Line-Point Intercept transects across the diagonal of each of the 6 blocks that will be seeded for Experiment 1 this fall. Use protocols similar to those for last year’s pretreatment characterization, but collect line-point intercept data only; do not collect biomass data. The cover data will be a total of twelve 25-m line-point intercept transects per site, with six transects in the herbicide area and six in the not-herbicide area.

**Measurements at the time of seeding**

Efforts are to characterize the seedbed environment into which seeds are placed.

**Variables**

1. **Seedbed soil moisture** – Randomly select 4 plots within each block. Using the handheld TDR probe, measure soil moisture near the center of each of the 4 plots.

2. **General observations** – Note general seedbed characteristics, such as dry/moist/wet soils, loose/compacted/crusted soil surface. Visually observe that seeds flow through flutes during the seeding to verify that seed is placed into the ground.

**Post-seeding measurements**

Screening efforts will focus on relative differences between accessions by evaluating establishment and performance indicators at 3 times a year for each of 2 years:

1. Early spring just after snow-melt (Jan-Mar)
2. Mid-spring (May)
3. Summer- peak biomass (Late-June to early July)

**Variables**

1. **Soil surface physical crust** – On the 1st sampling date of the 1st year, measure the soil physical crust. Each state will be responsible for conducting the slake test to measure soil stability (see end of document). Nicole DeCrappeo will determine the following: (1) the type of
crust (biological, physical, or chemical); and (2) characteristics of the physical crust (shear strength of soil surface using quantitative or qualitative methods).

2. Plant frequency (target species only) - A 3’ x 3’ steel frame (see figure below) will be placed in 2 random, non-overlapping locations within a subplot with the center of the frame centered on the drill rows. The outer 2 feet of each plot will not be included in sampling. The frame (quadrat) is divided into 9-1 ft² sections. Absence or presence of seeded seedlings or plants in each of the 9 sections will be recorded. Quadrats will be placed in the same location for all 3 sampling dates. Two corners of the quadrat will be permanently marked (rebar works well) so that the metal frame can easily be placed in the same location and so that the quadrat location is visible in order for personnel to avoid stepping in the quadrat area. Attach a metal label numbered 1, 2, 3, or 4 to the top left corner post to designate each quadrat so that the same quadrat can relocated in the future.

3. Plant density (Categories: (1) target species, (2) cheatgrass, and (3) all other species) – (1) The number of drill-seeded seedlings in 3 of the quadrat sections will be counted and recorded. Shaded portions of the quadrats represent these sections. (2) The outside perimeter of these 3 sections will have marks ground into the metal at 1, 2, and 3 inch intervals. Thin metal rods can be placed on these marks to form 1, 4, or 9 in² sections for counting the density of cheatgrass. The small 1 in² area may be appropriate at sites where cheatgrass is extremely dense. The 9 in² area is likely appropriate for sites where cheatgrass has a lower density. Be sure that the area of the mini-section used to determine plant density is recorded. These mini-sections will be located in the lower left-hand corner of the 3 quadrat sections. (3) Finally, count the number of ALL other species in the 3 quadrat sections, determining density over all species and NOT by individual species. However, compile a list of all other species, and record their abundance as L (low abundance, 1 individual in the quadrat section), M (medium abundance, 2-10 individuals), or H (high abundance, >10 individuals). Use the appropriate area (entire quadrat section if other species are rare or a mini-section if other species are more abundant) for determining the density of all other species, and be sure to record the area used for all other species.
4. **Biomass (Categories: target species, cheatgrass, and all other species)**—Six 1 ft$^2$ sections, centered on a drilled row, will be randomly selected at the first sampling date in the first year of sampling, and then the 4 corners of each biomass plot will be flagged so that personnel avoid stepping in the plots. Biomass will be determined for categories of species: (1) target species; (2) cheatgrass (and medusahead if present); and (3) all other species. At the time of peak biomass during the 1st year after seeding, compile a species list for all other species and record an ocular estimate of abundance divided into the same 3 abundance classes used for density (i.e. L, M, or H) on 3 of the 6 plots. Then, biomass will be clipped with hand shears at ground level. Biomass samples will be bagged and put on ice until they can be separated into the 3 species categories. Once separated into species categories, biomass will be oven dried at 60 °C for 48 hours, then weighed to determine dry mass. Biomass samples will be collected at peak biomass during the 2nd year after seeding from the remaining three 1 ft$^2$ sections in the same manner as above (i.e. by the 3 species categories and with a list of species for “other species” along with the ocular estimate of abundance).

5. **Plant survival measurements (target species only)**—Four individual drill-seeded plants within each of the same quadrat sections used for density measurements will be marked with a colored wire loop placed at the base of the plant. On the first sampling date, mark as many individual plants as possible, up to 4 plants. If less than 4 plants are present on the first sampling date, then mark additional plants on subsequent sampling dates until a total of 4 plants have been marked. Survival of these 4 marked individuals will be observed on each sampling date and used for repeated measurements of plant survival.

6. **Reproductive success measurements (target species only)**—Both vegetative and sexual reproductive success will be evaluated at peak biomass in each year. For each of the 4 plants used for the survival measurements that are marked with colored wire loops and (see above), record the total number of daughter tillers and inflorescences. 

*Special note for rhizomatous grasses:* Initially, daughter tiller production for each marked plant should be easy to distinguish because daughter tillers should be within 1-2 cm from the parent plant. However, at some point in time daughter tillers will be produced further from the parent plant. When daughter tiller production is at 3-5 cm from parent plants, then the sampling strategy will need to change to a complete census of the quadrat section. For the complete census, record the total number of tillers and inflorescences in the drill row for each of the 3 density quadrat sections with marked plants. Enter that data into the column for Plant 1 on the data sheet. Then count the total number of tillers and inflorescences outside the drill row, and record that number in the column for Plant 2. Write “Total census” in the columns for Plants 3 and 4. Note that the number of tillers outside the drill row divided by the number of tillers in the drill row will be used as an estimate of daughter tiller production (i.e. vegetative reproduction).

7. **Soil moisture**—Measure soil moisture with the handheld TDR probe in the center 1 ft$^2$ section of the 3’ x 3’ quadrat. Measure soil moisture adjacent to but not directly in the drill row and repeat measurements on each sampling date.

8. **Photos**—Take a photo of each block on each sampling date plus an overview of the entire experiment. In each case, standardize the location of each photo and be sure that the horizon is similar for all photos.
9. Miscellaneous – Exact dates for monitoring these plots likely will vary between sites and States. Appointed coordinators will be responsible for keeping up on the status of these seeded plots and relay this information to T. Monaco so that monitoring dates can be chosen or predicted for these sites.

**Measuring soil stability—Slake Test**

Materials needed: complete soil stability kit, sampling scoop, distilled water

The soil should be **air-dry** when performing this test. If the soil is not dry, collect crust fragments as described in Step 1 and allow them to dry.

1. With the wide end of the scoop, remove litter and any organic duff from above and the soil immediately in front of the surface sample you intend to retrieve. With the small end of the scoop, carefully remove a small piece (6 mm, about the size of a pencil eraser) of the soil surface. Be careful not to shatter the sample while removing. Collect a sample from near the center of XX plots in each of the 6 blocks per site.
2. Using two sample boxes, one with sieves and one without, fill compartments with water in the box without sieves. The water should be 2 cm deep and at approximately the same temperature as the soil.
3. Place a soil fragment in each sieve basket in the dry box, then at timed intervals of 15 seconds, lower a soil fragment in its basket into a compartment filled with water.
4. Observe the soil fragment for **30 seconds**. Refer to the stability class table below to determine if the stability class is 1, 2 or at least 3.
5. After five minutes in the water, raise the basket out of the water, then lower it to the bottom. It should take one second for the basket to clear the surface and one second to return to the bottom.
6. Repeat immersion four times (total of five immersions). Refer to the stability class table below to determine classes 3 through 6.

Soil stability is rated according to the time required for the fragment to disintegrate during the five-minute immersion and the proportion of the soil fragment remaining on the mesh after the five extraction-immersion cycles. The higher the stability class, the more stable the soil surface.

<table>
<thead>
<tr>
<th>Stability class</th>
<th>Criteria for assignment to stability class</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Soil too unstable to sample (falls through sieve)</td>
</tr>
<tr>
<td>1</td>
<td>50 % of structural integrity lost within <strong>5 seconds</strong> of insertion in water</td>
</tr>
<tr>
<td>2</td>
<td>50 % of structural integrity lost <strong>5 – 30 seconds</strong> after insertion</td>
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<tr>
<td>3</td>
<td>50 % of structural integrity lost <strong>30 – 300 seconds</strong> after insertion or &lt; 10 % of soil remains on the sieve after 5 dipping cycles</td>
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<tr>
<td>4</td>
<td>10 - 25% of soil remaining on sieve after 5 dipping cycles</td>
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<tr>
<td>5</td>
<td>25 - 75% of soil remaining on sieve after 5 dipping cycles</td>
</tr>
<tr>
<td>6</td>
<td>75 - 100% of soil remaining on sieve after 5 dipping cycles</td>
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