ENH1090

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Methodology for Wetland Seedbank Assays[1]

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Knowledge of the soil seed bank composition can contribute to predictive and historical understanding of plant community composition. Possible applications include:

1. determining likely species composition following a disturbance,
2. characterizing local weed population dynamics,
3. predicting the potential contribution of the seed bank to restoration of a target plant community, and
4. determining historical plant community composition.

The seed bank may differ from the current field population by containing species not currently found in the field population, or by having species occur in very different numbers than in the field population. One reason for this difference is that some species produce substantially more seeds than others; therefore, the seed bank is often dominated by a relatively small number of species. Species that are more likely spread vegetatively may have few or no seeds present in the seed bank. Another variable affecting species availability in seed banks is the length of time that seeds persist and remain viable. Viability can range from days to decades.

Despite differences between the seed bank and existing vegetation, the seed bank is an indication of the potential species composition at a site. In a restoration context, knowing which species are available in the seed bank can help natural resource managers make decisions about what species may need to be planted after a disturbance in order to have the desired species represented.

The purpose of this publication is to summarize standard techniques for wetland seed bank assays, including greenhouse set-up, sample collection and sample processing. Environmental conditions and "ponded" bench construction are presented first since these must be addressed prior to sampling (sample storage time may need to be minimized). The methodologies below are applicable in any climate, but care has been taken to mention specific considerations and references for warmer climates (particularly Florida).

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Environmental Conditions for Seed Bank Assays

Temperature and photoperiod are two environmental factors important to plant germination and growth (Baskin and Baskin 2001). When running a seed bank assay, make sure that the greenhouse (or other experimental site) and field site have similar light cycles and temperature ranges during the period of time that the assay is conducted (Harwell and Havens 2003, Smith et al. 2002, Williges and Harris 1995, Looney and Gibson 1995). Mimicking field conditions in the greenhouse will facilitate seed bank emergence that is typical of field conditions, and will also limit confounding variables in the assay.

If the temperature in the assay location is different from that in the field location, the difference should be minimized with heating or cooling (see Mahaney et al. 2004). Maintaining cooler temperatures can be a challenge for greenhouses during warmer months. If climate control is not available, cooling approaches can include a combination of an evaporative cooling system, and overhead and wall ventilation fans. During colder periods, maintain inside air temperature by covering evaporative cooling pads with an interior plastic sealing flap. This flap prevents most of the cooler outside air from entering the greenhouse, but allows for air exchange. Heating approaches can include an overhead heater tube connected to a furnace, and circulation fans.

If the photoperiod or light intensity differs between the greenhouse and the study site, augment natural light with artificial lighting (use a light meter to compare light intensity in the field and greenhouse). For example, seeds transported from Florida to Iowa received additional light from high-intensity bulbs matched to the photoperiod of the Florida field site (van der Valk and Rosburg 1997). Harwell and Havens (2003) used fluorescent tubes with wattages reflective of light data taken in the field to provide natural photoperiod and light levels to their submerged, aquatic vegetation seed bank assay. Natural photoperiod was maintained and natural light conditions achieved with the use of supplementary lighting in a number of seed bank studies (Mahaney et al. 2004, ter Heerdt et al. 1996, Boedeltje et al. 2002, and Combroux et al. 2001 and 2002).

In order to monitor seed bank assay conditions in the greenhouse, temperature and relative humidity data can be taken regularly (i.e. every 15 minutes) using a HOBO (temperature/relative humidity data logger). The HOBO should be enclosed in a solar radiation shield to protect it from solar radiation and reflected heat, and improve the measurement accuracy.

Ponded Benches Provide Optimal Wetland Emergence Conditions (for Saturated Soils)

One method of creating wetland conditions in a greenhouse is to construct a "ponded" bench for the emergence assay. To create your own ponded bench from an existing greenhouse bench:

1. Level an existing bench. Small differences in bench elevation can result in large differences in the hydrology of the trays in the benches. These differences will confound the assay.

2. After the bench is leveled, cover the entire top surface of the bench with plywood (Figure 1A).

3. Create a lip around the perimeter of each bench by attaching 2-inch by 4-inch boards to the surface (Figure 1B).

4. For bench drainage and cleaning, drill three holes through the plywood along the long side of the bench. The holes should be the same size as the drains being installed, and should be positioned near the edge of the bench. Drains and stoppers can be purchased at any hardware store.

5. Line the bench with a single sheet of ethylene propylene diene monomer rubber (EPDM) that is larger than the surface area of the bench by a few feet in all directions (Figure 1C and D). EPDM can be purchased in various sizes and shapes from internet sites specializing in pond liners and water gardens.

6. Mark the underside of the EPDM at the places where the drains are located. Make a vertical and horizontal slit in the EPDM that is smaller than the drain to be installed. Lay the EPDM back in place. With the EPDM acting as a washer, the drain can be screwed together and plugged with a stopper to create a water-tight seal.

7. Fill the bench with water. An automatic stock tank siphon float valve can be modified and installed on the bench to automatically maintain water at the desired level (Figure 1E). The valve supplies water to each bench through a garden hose coupled to a back-flow preventer.

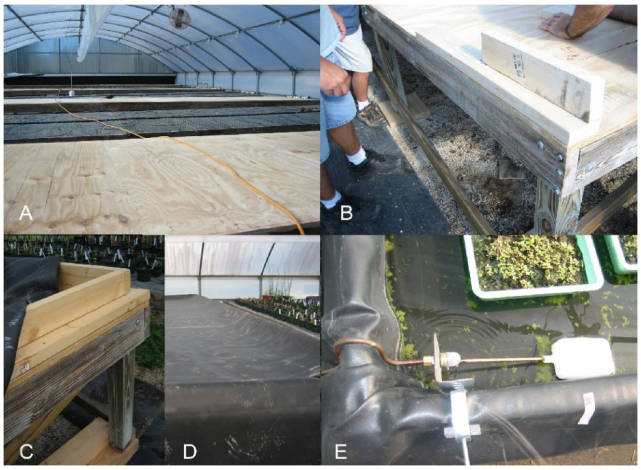

Figure 1. Construction of a "ponded" bench (A, B, C and D) and the float system (E) used to maintain constant water level. Credits: Nancy Steigerwalt.

Seed Bank Sample Collection

A stratified random sampling design is commonly used for seed bank sampling when differences in the seed bank are anticipated (e.g. vegetation zones, hydrologic zones, soil zones, land-use history etc.) (van der Valk 1978, Williges and Harris 1995, Poiani and Johnson 1988). For stratified random sampling, divide the study area into smaller units (subpopulations) representing anticipated differences, and collect samples randomly within each smaller unit (McCune and Grace 2002).

To account for the high spatial variability characteristic of many seed banks, it is preferable to take many small samples rather than a few large samples (Roberts 1981). Also, a number of small samples can be collected and composited (Bigwood and Inouye 1988); for example, five cores collected within a 5-square-meter area will generally yield more reliable data than two larger cores collected from the same measured area.

A tulip bulb planter is frequently used to collect seed bank samples; however, any sampler that allows for sampling a known volume will work. Seed bank samples are typically collected to a depth of 5 centimeters (Galatowitsch and van der Valk 1996, Poiani and Johnson 1988, Harwell and Havens 2003) because this region of the soil typically contains the highest density of viable seeds (Roberts 1981). However, it is not uncommon to take deeper soil cores to answer research questions.

Figure 2. Processing the sample: removing material that might vegetatively propagate from the sample (A), preparation of the tray (B), spreading the sample into the tray (C), and trays immersed in 4 cm of water (D). Credits: Photo A by Carrie Reinhardt Adams. Photos B, C and D by Nancy Steigerwalt.

Seed Bank Sample Processing

Seed bank samples should be kept separate in airtight bags and stored cold during transport. In order to keep seeds dormant until the emergence assay is set up, store samples in a dark refrigerator or cold room (Miao et al 2001, Leeds et al. 2002, Williges and Harris 1995, Hanselman et al. 2005, Looney and Gibson 1995). To ensure that all emerging plants are seed-source, remove any material that could vegetatively propagate (rhizomes, roots, and any remaining live plant tissue) and manipulate the soil through a screen (6.35-mm wire mesh is one choice) (Figure 2A).

Shallow polystyrene containers (e.g., Perma-Nest plant trays 20 x 20 x 6 cm) make excellent containers for seed bank assays and are available from a variety of merchants. If the containers do not have pre-drilled holes, drill 5 holes in the bottom of each tray to allow for water circulation. Fill each tray with a 2 cm porous layer using coconut coir fiber potting medium, sand, or absorbent rockwool (Figure 2B). Filling the bottom of the tray with porous material prevents gas formation and algal blooms that would otherwise occur in submerged trays (Boedeltje et al. 2002, Barko and Smart 1983). Add 2 cm of potting soil on top of the porous material. Spread the sifted seed bank sample on top of the potting soil layer evenly to a depth of 1 centimeter (Figure 2C).

Setup for Seed Bank Assay

Randomly distribute the trays in your greenhouse bench to avoid any bias in placement (Figure 2D). Not randomizing the trays can add a confounding variable to the assay. A common approach for seed bank assays is to set up two replicate assays, one with samples maintained at saturated water depths for emergent plants, and one with samples maintained at flooded water depths for submerged plants (Miao et al. 2001, Williges and Harris 1995, van der Valk and Rosburg 1997). Boedeltje et al. (2002) found that the 0 - 1 cm water depth ratio germinated both submerged and emergent plants, making the duplication unnecessary and considerably reducing the space and effort needed to complete the assay. If the research does not include emergent plants, the Boedeltje et al. (2002) method is most efficient. To follow this method, fill the bench with water to a depth within 0 - 1 cm of the trays' soil surface. Water level should be maintained within this ratio as it provides adequate water for seed emergence of both submerged and emergent plants (Boedeltje et al. 2002). Failure to maintain consistent water levels for all trays will confound the assay and has the potential to alter its outcome.

Seed from sources other than the sample (most likely greenhouse weeds) may contaminate the assay. To control for seed input, fill a number of trays with only potting medium (no seed bank sample) and place these trays randomly in the bench along with the seed bank trays. If plants emerge from the empty trays, you can determine the potentially contaminating species, and estimate the frequency of contamination.

Seed Bank Emergence and Identification

Remove emerged plants from seed bank trays when tentative identification or differentiation is possible (Figure 3) (Williges and Harris 1995). Remove unknown species from the assay, label all like specimens, and re-plant them in labeled pots. Grow unknown plants in a ponded bench until they are mature enough for positive identification. Flowers are usually necessary for identification, and flowering may be induced using fertilization or other means. Reserving all specimens that have tentatively been identified as similar allows for confirmation of identification as plants mature and become more easily identified.

Length of the Seed Bank Assay

In temporate regions, the majority of seeds germinate and emerge in less than 5 months (Mahaney et al. 2004, Combroux et al. 2001, Haag 1983). Due to the longer growing period in Florida, it is appropriate to conduct seed bank assays for 6 to 9 months (Leeds et al. 2002, Hanselman et al. 2005, Looney and Gibson 1995). Although emergence can be completed during this time period, the total length of the assay will likely be extended to include the time needed to grow unknown species to maturity for identification.

After the seed bank assay continues beyond the first few weeks, it is likely that some mold, algae, and/or moss may develop on the surface of the seed bank soil sample. This growth might act as a physical barrier to emergence. To minimize this problem, maintain the water level below the soil surface. Should significant mold, algae, and/or moss accumulate, periodically slice through the material with a razor blade and otherwise disturb the soil surface to provide space for seedlings to emerge (A. Frances, personal communication).

Figure 3. Plants emerge from the seed bank assay three months after set up (A), are removed from seed bank trays (B), and are recorded and discarded if identity is known. If identity is unknown, plants are repotted (C), and grown out in ponded or drip-irrigation benches (D). Credits: Nancy Steigerwalt.

Conclusion

A seed bank assay can provide valuable information regarding the viable seed population in the soil. Careful consideration of potentially confounding factors can prevent bias in seed bank assay results. Although seed bank assay methodology was initially developed in temperate environments, simple adaptations to common methodology allow them to be conducted in varying climates.

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