

Field protocol for sampling tissue from wavyleaf basketgrass patches

Questions? Please contact Dr. Carrie Wu

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Addresses to send wavyleaf samples during covid-19 pandemic:

Preferred - Home:

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Not checked as frequently - Work:

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Before collecting tissue, record the following to include with your samples:

- Collector's name
- Sampling date
- Location information, including GPS data if available
- Estimated size of wavyleaf basketgrass patch
- **IMPORTANT:** If you will be sending seeds across state lines, please first contact Carrie Wu (cwu@richmond.edu) so that you can include the necessary permits.

General info:

- When collecting tissue for genetic studies, the key is to keep the tissue from getting brown or moldy, and instead stay dry and green. **Storing leaves wrapped in paper (envelope, newspaper, or printer/notepad paper) or in a plastic bag containing a drying agent like silica gel are two ways to do this.**
- If the tissue is wet from dew or a recent rain, blot the leaves dry before collecting.
- Aim to collect at least 4-5 green leaves from a single plant. If possible, follow the stem back to the root, and collect leaves from other stolons (runners) that are connected to that same root. By including as much leaf tissue as possible from a single plant, we will have the best chance at successfully extracting DNA from that individual.

How many plants to sample:

- **Sample from plants about 1 m apart, and check that the stems aren't connected by runners.**
- In small patches (approx. 1 m), try to collect one plant from each of the cardinal directions (four points on opposite edges of the patch). If all plants appear to be connected, only collect from one plant in the patch.
- In larger patches (> 1 m), collect plants at least 1 m apart to prevent resampling from the same individual. If possible, collect tissue from up to 15-20 plants in these large patches, which will help us to be able to estimate the genetic diversity of the patch.

Preferred collection method for leaf tissue:

• **Supplies needed:**

- Ziploc plastic bags
- Smaller paper envelopes (coin envelopes or letter-sized envelopes) or pieces of paper (newsprint, paper bags, printer paper, or notepad paper will work)
- Silica gel (ideally)
- Pens/pencils for labeling

In the field:

- Collect 4-5 leaves (at least) from a single plant, and place into a coin envelope or regular-sized envelope. If no envelope is available, fold the tissue into paper to press the leaves from the plant into a bundle that will stay separate from other collections.
 - Try to collect nice green leaves that aren't brown or shriveled.
 - It's okay to include the stem.
 - Label the envelope with a unique identifier that includes site name
 - For example, two individuals collected from a site called "University of Richmond (UR)" can be labeled "UR-1" and "UR-2".
- Put labeled envelope into a Ziploc bag that contains approximately 3-4 tablespoons of silica drying crystals (**see next page), as shown in **Figure 1** below. Multiple envelopes can be placed into a single Ziploc bag.



Figure 1. Leaves from single plants are collected in separate coin envelopes, then stored in a larger bag with drying crystals



Figure 2. Leaves from one plant collected directly into small Ziploc bag containing drying crystals, which is placed inside another Ziploc bag.

Alternate collection methods:

- **If you do not have access to envelopes**, leaves collected from a plant can be placed directly into a small plastic bag containing silica gel (**Figure 2** above). Be sure to label the plastic bag! As with the coin envelopes, you can then put multiple small plastic bags together into a larger bag to keep them together.
- **If you do not have access to silica gel**, leaves can be collected in paper envelopes, lunch bags, pieces of newspaper, or other absorbent (not waxy) paper that is folded closed, similar to pressing for herbarium samples, if (1) collections will be delivered promptly for preservation by silica gel or freeze-drying, and (2) **absolutely no seeds are included**. Please place such samples inside a plastic bag before placing in the mail.

****Sources for silica gel drying crystals**

Silica gel drying crystals can be found in the floral section of most craft stores, including AC Moore, Michael's, and Walmart. Similar products online include the following:

https://www.amazon.com/gp/product/B00BSYHGXQ/ref=s9_dcacsd_dcoop_bw_c_x_2_w

<https://www.amazon.com/Panacea-60102-Silica-Crystals-1-5-Pound/dp/B001E5U3QQ>

In a pinch, you can also use those “DO NOT EAT” silica desiccant packets found in vitamins, some boxes of shoes, etc. The caveat is that since we don't know how much moisture those packets have already been exposed to, their ability to absorb moisture from your sampled leaves is questionable, and shouldn't be relied upon for long-term storage.

To collect seeds:

- **Be EXTREMELY careful about collecting seeds, to be sure that you do not leave the patch with seeds attached to your clothes or gear. Check, double check, and then check again before you leave the area!**
- If collecting plants with seeds, the seeds need to be **double bagged** inside two Ziplocs.
- To collect inflorescences (stems with seeds), simply pinch off or use scissors to cut off the inflorescences and place them inside a paper bag, envelope, or plastic bag. The bag or envelope in which you collected the seeds **must be double bagged** in sealed Ziploc plastic bags.
- Please indicate whether the bag contains seeds just from a single plant, or from multiple inflorescences from that site.

Please send me any receipts that you need reimbursed for collecting supplies or shipping expenses. *Thank you for your help!*