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HYBRID WATERMILFOIL GENETIC TESTING PROTOCOL

Hybrid crosses between native and non-native species can result in management challenges. Hybrid species tend to have greater genetic diversity, which may make them more resilient to management techniques. Laboratory and field studies have shown that some strains of hybrid watermilfoil, a cross between non-native Eurasian watermilfoil (*Myriophyllum spicatum*) and native northern watermilfoil (*M. sibiricum*), have reduced sensitivity to several commonly used aquatic herbicides compared to pure-strain Eurasian watermilfoil. Identifying potential hybrid strains can help resource managers pursue an effective management strategy.

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The Montana State University (MSU) Department of Plant Sciences and Plant Pathology offer genetic identification services for watermilfoils. If you suspect a lake may have hybrid watermilfoil, first check the Wisconsin Department of Natural Resources (DNR) Lakes Page to see if hybrid watermilfoil has already been verified. If it has not been verified and you would like to have it genetically tested, reach out to your regional DNR AIS biologist to coordinate sample collection and shipment. Funding may be available to help pay for genetic testing; your regional AIS biologist can provide more information on potential funding opportunities.

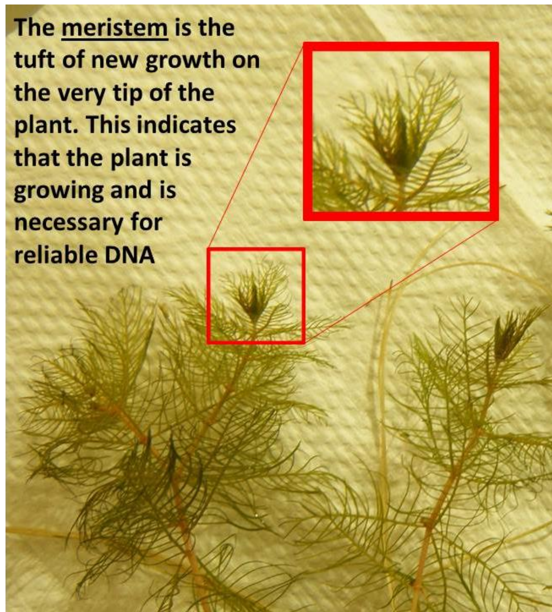
There are two types of genetic tests available: taxon level and strain level. The taxon level test will identify the species of each sample (i.e., it will determine if each sample is non-native Eurasian watermilfoil, native northern watermilfoil, or a hybrid of the two species). The strain level test will identify the species and will also provide a DNA fingerprint for each sample, which allows the lab to identify the strain. Ongoing research has shown that certain strains may be resistant to herbicide treatments, so identifying the strain(s) present in a lake may allow resource managers to make more informed management decisions in the future as research progresses. Although the estimated processing time for the strain level test (2-3 months) is longer than the processing time for the taxon level test (2-3 weeks), the costs are the same (~$50/sample). Unless a quick turnaround time is necessary, which your regional AIS biologist can help determine, we recommend that the strain level test be performed.

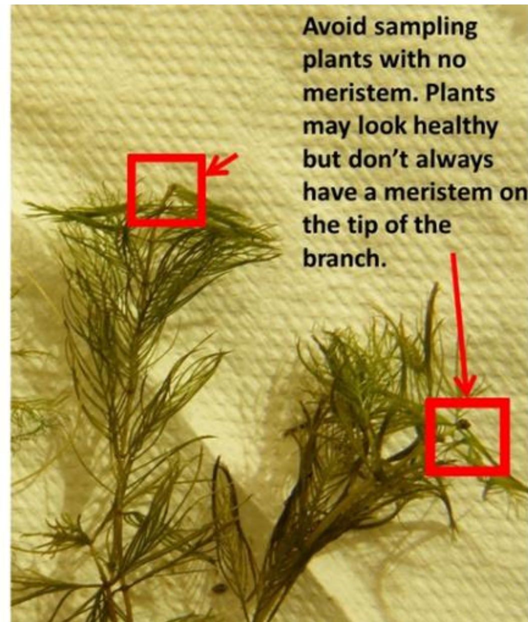
Before collecting any samples, contact your regional AIS biologist to develop a sampling and shipment strategy. To accurately estimate the genetic diversity within the lake, your regional AIS biologist will advise you to collect milfoil samples from several different locations (typically 3[*]) across the lake. Samples can either be sent to the lab dried or live (i.e., without drying out the plant). Note that the DNR will generally only provide funding for dry sample shipping due to the high cost of shipping live samples. Additionally, the lab prefers receiving and working with dried samples since their analysis is not as time sensitive to coordinate as it is with live plants. DNR staff may be able to provide drying supplies or dry/ship samples for you. Below are instructions on how to collect watermilfoil samples for analysis, as well as the dry and live shipping techniques.

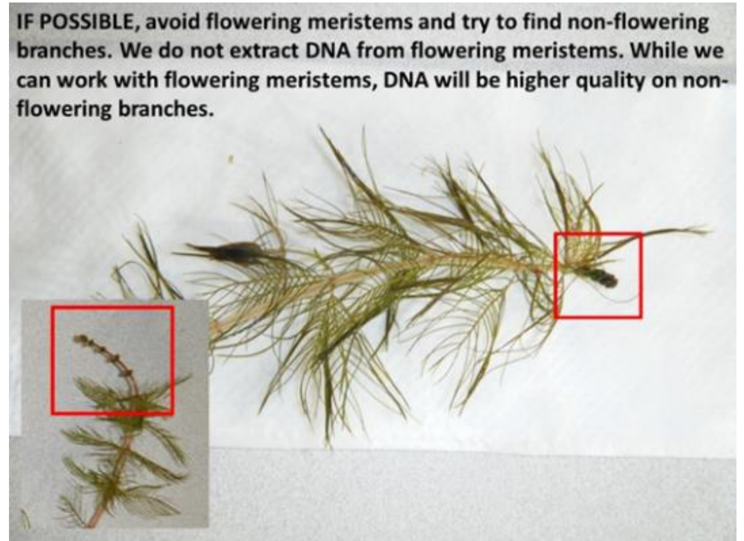
[*] MSU recommends collecting 20 samples to fully characterize the strains present in the lake. However, due to the high costs (~$1,000) associated with processing 20 plant samples, the DNR will generally only provide financial support for the shipping and analysis of 3 samples.

SAMPLE COLLECTION

1. Collect 4-6 inches (for dry shipping) or 6-10 inches (for live shipping) of plant meristem (the growing tip at the top of the plant).
    - Be sure that each stem collected for ID has a healthy growing meristem on its tip. The meristem has the highest quality of DNA and yields the best results.
    - Only collect one plant sample per location within a lake.
    - Avoid sampling plants without meristems, plants with flowering spikes, and meristems from the same plant (i.e., connected by branches).







2. Rinse off any debris or algae using gentle agitation in tap water to make sure the meristem is clean.
    - DNA from any other algae or debris cannot be rinsed off after the plants are dry and will contaminate the sample when they are processed.

SHIPPING OPTION 1 (PREFERRED OPTION): DRY SHIPPING

IMPORTANT: Plants should be placed in silica within a few hours of collection and dried within 24 hours of collection to properly preserve the DNA; the sample will degrade if it sits in water for too long, which lowers the DNA quality and may make the sample unsuitable for genetic analysis. We recommend keeping plants in a cooler while sampling in the field, and then drying them immediately after sampling. Dry samples must remain in silica beads through the entire duration of storage as well as during shipping to prevent any potential rehydration before processing. Rehydration of the plant will result in degradation of the DNA.

Materials Needed:

- A watertight dry box large enough to fit all plant samples.
- Silica beads: 20 grams, or approximately 2 Tbs, per plant sample, plus enough to cover at least 1 inch of the bottom of the watertight dry box.
  - Silica beads that change color to indicate saturation are necessary to monitor for moisture, however they tend to be more expensive than plain silica beads. You can use a mixture with a 1:3 ratio of color-changing silica beads to plain silica beads.
  - Silica beads can be ordered online from many major online retailers. They may also be available at home improvement stores.
  - Saturated silica beads can be re-used after drying in an oven at low temperatures (~200 ^{0}F) for 20 minutes to 2 hours, depending on the type of beads used; the beads will be fully dried when the color-changing beads revert to their original color.
- Small paper envelopes (approximately 5½” x 3⅛”): 1 per plant sample.
- Quart-sized resealable plastic bags: 1 per plant sample.

Instructions:

1. Blot plants dry using a paper towel.
   a. Blotting plants dry removes excess water and helps keep silica beads from becoming saturated too quickly.
   b. Use a clean paper towel with each plant stem to help prevent contamination.

2. Place one plant stem individually in a small (5½” x 3⅛”) paper envelope, making sure that the plant tips are not in contact with the rest of the stem, and seal the envelopes.
   a. Keeping the tip clear of the rest of the plant reduces the amount of contamination when removing the tip for analysis.
   b. **IMPORTANT:** Do not put silica beads directly in the paper envelope with the plant! Silica beads should never come in direct contact with the plant tissue. This will damage the plant tissue and contaminate the beads, which means they cannot be reused.

3. Label both the paper envelope and a quart-sized resealable plastic bag with:
   a. Lake name
   b. County
   c. State
   d. Location within the lake (i.e., site number or lat/long coordinates)
   e. Collection Date

4. Place one sealed paper envelope individually in the plastic bag with around 20 grams (or 2 Tbsp) of silica beads.
   a. Keep the envelope and plastic bag as dry as possible to avoid saturating the silica beads too quickly.

5. Force as much air as possible out of the plastic bag before sealing it.
   a. Removing the air will make it possible to store more bags in one watertight container. It will also help prevent the beads from saturating too quickly.

6. Place all bags in the watertight container with around 1 inch of silica beads on the bottom.
   a. This ensures that the plant does not rehydrate during storage.

7. Check silica beads for saturation after 24 and 48 hours, and then every 3 to 4 days until plants can be sent to the lab for genetic identification.
   a. Silica beads should be replaced in any bag that has greater than 50% saturation (i.e., more than 50% of the color changing beads have changed color).
   b. Plants can be stored in the watertight container at room temperature for 2-3 months before shipping.

8. Fill out the chain of custody form with the collection information for each sample and send it to your regional AIS biologist. Your regional AIS biologist will then provide additional instructions on coordinating shipment to the lab.

9. [**DNR STAFF ONLY**] Enter the chain of custody information into SWIMS using the data upload protocol.

SHIPPING OPTION 2: LIVE SHIPPING

IMPORTANT: Plants should be collected, processed, and shipped either the day of collection or the day after collection using this method. The longer that plants sit in the refrigerator or the mail, the less likely it is that the DNA will be properly preserved; the sample will degrade if it sits in water for too long, which lowers the DNA quality and may make the sample unsuitable for genetic analysis. Plants need to be sent using overnight shipping in a cooler that you do not need returned; **MSU will not ship your cooler back to you.**

Materials Needed:

- Gallon-sized resealable plastic bags: 1 per plant sample.
- Paper towels.
- Insulated package for shipment (e.g., plastic or foam cooler).
- Cold packs: at least 1 per cooler.

Instructions:

1. Lay each stem out on a damp (NOT WET) paper towel.

2. Place another damp (NOT WET) paper towel on top of the plant and gently roll it up, like a burrito.

3. Place the paper towel roll on the bottom of a gallon-sized plastic bag.

4. Label the bag with:
    - Lake Name
    - County
    - State
    - Location within the lake (i.e., site number or lat/long coordinates)
    - Collection Date

5. Carefully pack the bag(s) of plants into an insulated package (e.g., plastic or foam cooler) with one or more cold packs.
    - Be careful to cushion the plants from the cold packs so they do not crush the plants.

6. Fill out the chain of custody form with the collection information for each sample and send it to your regional AIS biologist. Your regional AIS biologist will then provide additional instructions on coordinating shipment to the lab.

7. [**DNR STAFF ONLY**] Enter the chain of custody information into SWIMS using the data upload protocol.