

### **PROTOCOL: Leaf Collection and Handling in the Field.**

#### **(i) Equipment**

- Tree climbing equipment
- Pole pruners
- Shotguns and ammunition
- Bows and arrows
- Slingshots
- Large coolers
- Tarps

#### **(ii) Consumable materials**

- Large plastic bags (2 gallon size, Ziploc freezer bags work best)
- Small Ziplocs (quart-sized)
- Paper towels
- Labeling markers
- Frozen water bottles
- Leaf clippers and scissors
- Newspaper
- Leaf punching supplies – cork borers (7 mm diameter), rubber mats, labeled tinfoil squares (2.5" x 2.5")
- Pantyhose

#### **(iii) Field sample collection and handling**

- Collect 2-3 tree branches from the sunlit portion of the canopy using climbing, pole pruning, roping or shooting techniques which meet the local site requirements.
- Immediately select 6 mature leaves for spectroscopy (measurement detailed in 'Foliar spectroscopy protocol'), ensuring the branches are kept well out of direct sunlight.
- From these same leaves punch at least 30 disks out of each leaf using a sharp cork borer, avoiding the mid-rib and any large veins.
- If leaves are too tough or fibrous (e.g., Arecaceae), cut small squares of similar size to the disks with scissors.
- Place each set of 30 punches in a separate pre-labeled tinfoil squares (set 'A'). Augment each set with additional leaves if necessary to reach 30 disks.
- Fold tinfoil packets and place in a small Ziploc in a cooler with frozen water bottles to keep cold.
- Pick an additional 6 leaves of similar quality and repeat the punching process for set "B" tinfoils.
- Collect tinfoil packets in groups of 10 specimens in the quart Ziplocs.
- If vouchers have not already been taken, select 2 branches from those collect for voucher specimens. Make sure that important characteristics of the plant are collected for voucher, such as fruit, flower, dimorphism, etc.
- Place each branch in a newspaper 'folder' labeled with the specimen code and species code if known.
- Put the remainder of the leaf sample still attached to the branches into a pre-labeled large plastic bag containing a damp paper towel.
- Breathe into the bag to humidify and to potentially close the stomata on the leaves.
- Remove excess air without crushing the leaves and seal the bag.
- Store bags in large coolers with frozen water bottles or blue ice until transport back to the lab.
- Once all the vouchers are collected for the day, seal them in a plant press.

(iv) **Sample stabilization at the field site laboratory**

- Once back at the mobile lab, immediately introduce sets of frozen leaf disks to labeled stockings, and then to a 'dry-shipper' containing liquid nitrogen (or store them on dry ice) until they are transported to the Spectranomics Laboratory in Arizona.
- Begin measurements for specific leaf area (SLA) and water on bulk samples (detailed in 'SLA and water protocol').
- Place plant press in 65 °C oven for drying for at least 72 hours.