SAMPLE COLLECTION PROTOCOLS FOR CALLERY PEAR LEAVES

We are grateful that you are willing to assist us in our Callery Pear (*Pyrus calleryana* Decne.) population genetics study. Your effort will directly contribute to the success of the undergraduate research project undertaken by Ms. Lizbeth Evette Chavez at the University of Tennessee. Ms. Chavez's project is conducted in collaboration with Clemson University and will help us understand the genetic diversity present among Callery Pear trees that were originally imported from its native China to help develop fire blight resistance in the common pear (*P. communis*). A number of different Callery Pear cultivars are commercially available and individuals of these cultivars are self-incompatible, thus are reproduced asexually via grafting and rootstock shoot cuttings. Trees are, however, cross compatible when situated near different cultivars and wild accessions. When trees are cross-pollinated, they will yield viable seed. Trees can also reproduce if suckers on above-ground portions of the rootstock are allowed to mature to a produce flowers, and by shoot generation from below-ground, rootstock portions. Consequently, escapees of these non-native deciduous, ornamental trees have become economically and environmentally important across the eastern United States as an invasive species.

Callery Pear Plant Material Collections:

A. At each location, collect 10 leaves from each of 10 different Callery Pear trees*. Each site should be no more than about an acre (about the size of a football field) in size.
B. Site locations (containing 10 different trees) should be 15 miles apart minimum if collecting in different locations in your state (3 to 5 site locations per state are our experimental goal).
C. Place each tree's leaf samples together in a single paper envelope (the leaves will be dried).
D. Label each envelope with the GPS coordinates for **each tree**.
    a. (e.g. TN_KN_1 and TN_KN_2 for 2 different TN locations located in Knox County).
E. Place all 10 envelopes per location (each with ~10 leaves per tree) in a brown paper bag.
    a. Paper bag and contents can be air dried; don't store in sealed plastic bags.
F. Ship samples to the address provided below.

* *Trees used for LEAF SAMPLING should represent individuals from naturally-grown populations (e.g., "volunteer seedlings") and represent open-pollinated specimens, rather than trees that were planted in landscapes that may be horticultural clones (cultivars) or transplanted from nursery stock imported from external locations. We request that trees suspected to be (trans) plantings of commercially-grown cultivars should be excluded from sampling – in other words, if it's planted in a row, or yard, or any other "domesticated" setting, please don't sample it – we are after trees growing in non-landscaped areas.*

Callery Pear Leaf Collection Details

Callery Pear leaves start to appear about a one to three weeks after flowers start to open in the spring. Ideally, young, disease-free leaves will be collected for analysis. We anticipate that once suitable sites are located, it will take between 30 minutes to one hour to collect leaf samples from each of 10 individual trees* per location, and to record/save the coordinates for each tree.

Callery Pear Identification:

1. A small (16 to 25 ft tall) tree with conical to rounded crown that is often used as a popular ornamental tree across the Eastern U.S. (Fig. 1).

2. The white, five-petaled flowers are about 3/4 to 1 in. in diameter with an unpleasant odor in early spring. They appear before the leaves expand fully (Fig. 1) and in many places are one of the first trees to produce white flowers in the spring.
3. Leaves are simple, alternate and oval, 1½ to 3 in. long; dark green in the summer, and orange or purple in the fall (Fig. 2).
4. Small fruit is round, green/brown, and inedible (Fig. 2).
5. Branches and twigs have brown, rough bark (Fig. 3).

Figure 1: Wild Callery Pear tree (upper left) and close up of white flowers (upper right) and commonly planted landscaping trees (bottom). For this project, we would like to avoid these samples pictured in the bottom image. The flowers appear right out of the bark/branches Photo credits: Gary A. Monroe, David Coyle, and Andre Dabar.

Figure 2: Callery Pear trees are easily identified by their round-shaped leaves, which appear after the flowers. Newly emerged, young leaves are preferred for high quality DNA extractions. The fruit and flowers of native pear species (e.g. *Pyrus communis*) are larger than those of Callery Pear. Photo credit: wikipidia.org and missouriplants.com.

Figure 3: Callery Pear bark has rough, textured bark that is green or brown in color (left). The thorns (which can be over 3 in. long; middle and right) on the branches create significant problems for homeowners, farmers, and landscapers. Photo credits: David Coyle and walterreeves.com.

Items needed for leaf collections (will be provided for each collector, if needed):

- Pre-folded paper leaf-envelopes (i.e. mailing envelopes) (use 1 envelope for all 10 leaves per each tree)

- Brown paper Kraft bag (use 1 bag for all 10 trees at each location)
- Sharpie pen/pencil (for labeling envelopes and bag)

Leaf collection methods

1. Select 1 to 3 locations apart from each other (minimum 15 miles distance). Each location selected should contain 10 trees growing in a naturalized landscape, field, or right-of-way area. Disturbed areas (recently forested woodlots), roadside edges, rest stop grounds, churchyards & cemeteries (with permission), adjacent to railway borders, etc. are often excellent locations for finding natural seedlings and wild trees (Figs. 4-5).

2. For leaf samples, we are exclusively interested in trees that are **wild in origin** and must avoid named cultivars and clonal trees to protect the experimental integrity.

3. From each individual tree please randomly select 10 to 12 disease-free young leaves (Fig. 2). Leaves should be from at least 5 different branches on the same tree. Leaf samples can be stacked together and should be placed directly into a folded paper envelope (provided). In case they are wet, please separate leaves by placing the paper in between each leaf (see sample preservation). Use 1 envelope per tree and record the GPS coordinates for each tree directly on the paper envelope.

4. The 10 leaf sample envelopes (from all 10 trees within 1 location) can be kept together in one brown Kraft bag (provided if needed), on which the state and location information are written.

5. Please be sure to write the GPS coordinates on the sample packets and label the samples with your state, county, and location codes (ex. TN_KN_1, TN_KN_2 and TN_KN_3 for 3 different TN locations located in Knox County)

6. To record GPS coordinates for each tree, you can use your phone or GPS device:
    a. On your Android phone or tablet, open the Google Maps app .
    b. Touch and hold an area of the map that isn't labeled. You'll see a red pin appear.
    c. You'll see the coordinates in the search box at the top.
    d. Please record these coordinates on the envelope used to hold leaf samples ***from each tree***. In other words, once completed, each location should include different coordinates for each of the 10 sampled trees.

7. Please record if the sampled tree has thorns (see Fig. 3). These may be on the branches, stem, or on both branches and stem, and they often have buds near the end of the thorns. Simply write "thorns" or "no thorns" on each envelope.

Sample Preservation

Leaf samples have to be dried properly to assure high-quality DNA is available for extractions. To avoid mold growth, please try to dry out the samples as soon as possible. The paper bag can be left open in a well-ventilated area/countertop [exposure of bags to sunlight can speed drying times] and checked after 24 hours to ensure that leaves are drying. If not, you can put some paper towels in between the leaves to speed up the drying process.

Figure 4: Callery pears growing in a natural landscape, in this case along the side of a road. Photo credit: David Coyle.

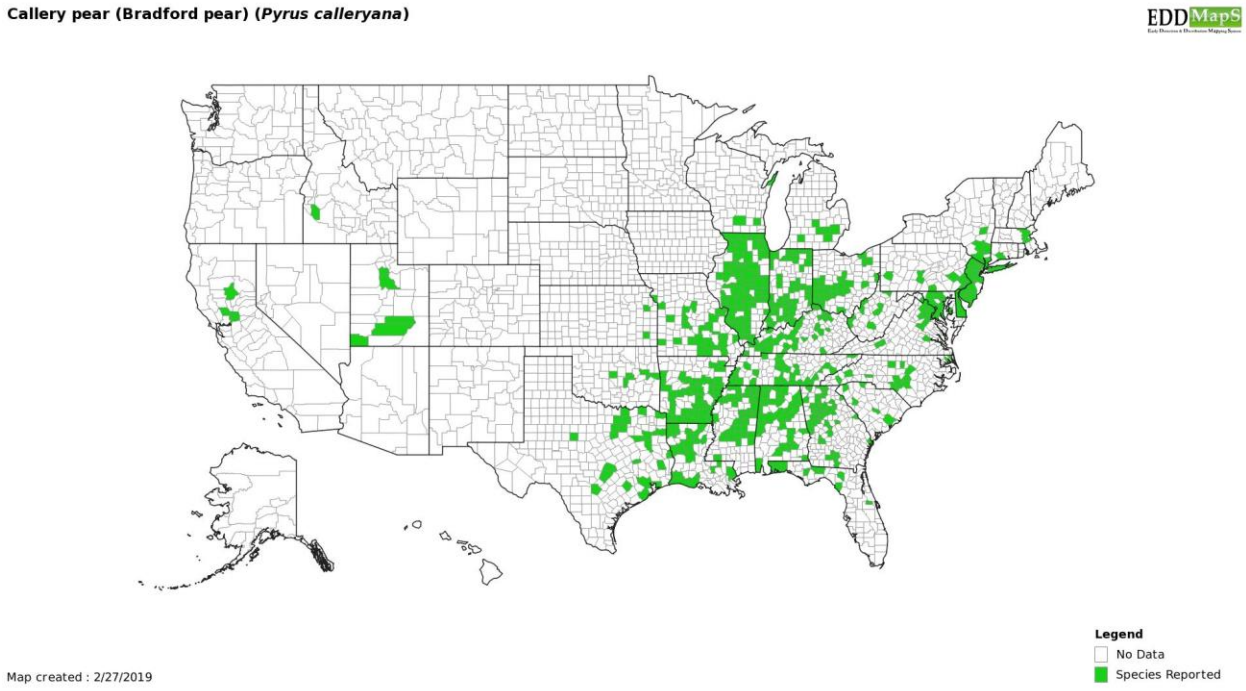

Figure 5: Callery pear distribution map. More info can be found at early detection and distribution mapping system (EDD MapS) website: https://www.eddmaps.org

Contact:

If you have any question about the collecting procedure or the project, please contact us:

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FedEx account number to be charged for shipping your samples to us: 183756184

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