Sampling Instructions for Avocado Sunblotch Viroid (ASBVd) Testing

Avocado sunblotch viroid (ASBVd) is a small (247 nt) pathogen that causes severe discoloration and disfigurement in both leaves and fruit. It spreads easily via grafting, contaminated pruning tools, pollen, seed transmission, and root grafting. Over time, infected trees can recover from their visible symptoms and become "symptomless carriers". While these carrier trees show no outward signs of sunblotch, they still maintain high viroid loads in their leaves, fruit, and seeds, and can readily spread the pathogen through their pollen. Because symptomless carriers typically suffer from low fruit set, using them to produce nurse seeds poses a significant risk for widespread viroid transmission.

Our laboratory utilizes highly sensitive droplet digital Loop-Mediated Isothermal Amplification (ddLAMP) for primary screening, with secondary confirmation performed via droplet digital Polymerase Chain Reaction (ddPCR) to ensure the highest diagnostic accuracy. Please follow the guidelines below to collect your samples.

Pricing Structure

- Batches of 20 or more samples: $25.00 per sample
- Batches under 20 samples: $30.00 per sample

(Note: One sample is equal to 1 bag containing 20 leaves or fewer.)

Please follow these guidelines carefully to ensure accurate diagnostic results. Proper sampling maximizes the chances of detecting the viroid if it is present.

1. Large / Mature Trees

Because sunblotch can be unevenly distributed throughout a large tree canopy, a thorough "walk-around" sample is required.

- **Leaves:** Walk entirely around the tree. Collect a total of **40 leaves** per tree, take **10 leaves from each of the four sides** (North, South, East, West). Be sure to sample leaves from both the **upper and lower** parts of the canopy. Pool all 40 leaves into a single bag for that tree.
- **Fruit:** If the tree has symptomatic fruit (or if you have specific concerns about asymptomatic fruit), collect **5 fruits from each side** of the tree (20 fruits total). Pool them together.
- **Flowers (Optional):** If you have specific concerns about flowering blocks, flowers can also be tested. Please contact the lab or include them in a separate labeled bag if needed.

2. Smaller Trees and Nursery Stock

For younger trees, smaller canopy sizes, or nursery settings, use the following leaf counts:

- **Very Small Seedlings:** Collect **2 leaves** total per tree.
- **Potted/Nursery Trees:** Collect **4 to 5 leaves** total per tree (similar to our standard nursery setup).
- **Semi-Mature / Smaller Established Trees:** Collect **10 leaves** total per tree (take **5 from the Southwest** side and **5 from the Northeast** side).

Packaging & Shipping Instructions

- **Segregation:** Keep samples from different trees in separate bags.
- **Bags:** Place all pooled samples (leaves or fruit) into clean, sealed Ziploc bags.
- **Labeling:** Clearly label each Ziploc bag with the Date, Tree ID/Row Number, and Variety using a waterproof permanent marker.
- **Shipping:** Send the bagged samples to our laboratory immediately. Keep samples cool (but do not freeze them) if there is a delay in shipping.