

Sampling Q Biotypes in Greenhouses

By Dr. Tim Dennehy

(circa 2005-2006)
revised 2016 by L.S. Osborne

Introduction

The Q Biotype Task Force has requested that its Technical Advisory Committee (TAC) recommend sampling procedures for surveillance of the Q biotype of *Bemisia tabaci* in 2005. These recommendations are intended for use by personnel in the 12 states in which surveillance is being recommended. The recommendations below are intended to provide guidance for personnel with limited familiarity with whitefly biology. Individuals with experience with sampling whiteflies may choose to modify these recommendations based on their knowledge.

Objective

At each location sampled, the objective is to collect a minimum of 10 and a maximum of 50 whitefly nymphs, pupae, or adults from plants distributed widely within the sample location.

Relevant biology

Parthenogenetic reproduction. *B. tabaci* is capable of reproduction without mating. Unmated females produce male offspring. A single female is capable of producing males that, upon maturity, can then inseminate the female and result in subsequent production of both male and female offspring by the female. Thus, introduction of a single female could result in establishment of the Q biotype at a location.

Clumped distribution. At low densities, whiteflies typically are highly clumped in distribution. Individuals within low density infestation foci are genetically related. Thus, it our objective to sample whiteflies from as many foci as practical within each location sampled.

Details

Sampling universe. In this case, the sampling universe is a greenhouse production facility. It is up to the sampler to define specific area sampled within this universe. To the extent possible, collections should be distributed among the Bemisia hosts produced at the location and within as many greenhouse units as practical. The more diverse the hosts and the greater the number of houses sampled, the better.

Sampling unit. A plant is the sampling unit. Whenever possible, no more than one whitefly should be collected from any plant and plants sampled should be at least 10 meters (i.e., paces) apart.

Sample. The ideal sample will consist of 50 individuals. The minimum number necessary to submit a sample for testing will be 10.

Stages to be sampled. Large nymphs (3rd or 4th instar), pupae, or adults may be collected in any combination.

Collection process. Adults may be aspirated off leaves and exhausted into microcentrifuge tubes or other vials. Nymphs and pupae should be excised from leaves using a scalpel or Xacto knife, with care given to limiting the amount of leaf material.

Hosts to be sampled. All hosts infested with *B. tabaci* are suitable for sampling. However, if choices must be made, hosts involved in international trade of propagation material should be a priority.

Preserving samples. All samples should be placed in 95 -100% ethyl alcohol that is free of any denaturants and placed into standard microcentrifuge tubes or other small vials. It is critical that the sample remain fully immersed in alcohol. Thus, attention must be paid to filling the container fully with alcohol and/or keeping the containers upright. Once in the laboratory, samples should be held in a conventional freezer prior to shipment to the analytical laboratory.

It should be noted that these instructions are for samples to be evaluated by Dr. Cindy McKenzie and other laboratories that are doing PCR and sequencing of the mitochondrial cytochrome oxidase 1 (mCO1) gene. The esterase electrophoresis conducted by Dr. Frank requires samples to be frozen, free of alcohol.

Shipment of samples. Ideally, samples should be shipped cold to the analytical laboratory via a one-day delivery service. The easiest way to do this is in a Styrofoam shipping container with Blue Ice or equivalent. At the very least, samples should be protected from exposure to heat.

Information to be submitted with samples. For samples to be tested with public funds, each must be submitted with the following information to one of the laboratories approved by the TAC:

Collector contact information (required)

- Collector's name
- Collector's organizational affiliation
- Office telephone number
- Mobile telephone number
- Email address
- Mailing Addresses

Sample information (required)

- Your sample identity code (please do not explicitly identify the location)
- Date collected
- State in which collection was made
- County in which collection was made
- Host plant or plants from which collection was made

Please circle the location information that applies

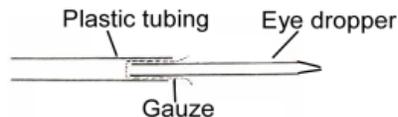
- Greenhouse
- ≤ 100 m of a greenhouse, but not in a greenhouse
- Other (please specify)

Site-specific information (optional but helpful)

- Approximately how many whitefly treatments were made to the typical greenhouse at this site in the past year?
- Which insecticides are most commonly used to control whiteflies at this site?
- Have whitefly control problems been more common in the past 12 to 24 months?
- Was there anything unusual or notable that prompted the collection being made at this site?

Equipment Specifications

- Aspirators. These can be made for a few dollars apiece. Eye droppers can be purchased from a drug store. Take the eye droppers to Ace Hardware or equivalent and purchase 24 inch lengths of Tygon tubing or equivalent that fits snugly over wide end of eye dropper. Purchase cheese cloth, gauze, or flexible screening of mesh diameter smaller than an adult whitefly. Cut into small squares of size appropriate to fit over wide end of eye dropper. Push tubing over wide end of eye dropper after placing square of cloth or screen over wide end.



- Alcohol. $\geq 95\%$ Ethanol. No denaturant. One example is the 100% ethyl alcohol that we can obtain through the University of Arizona Stores for \$11.52 per gallon. It is manufactured by Aaper (<http://www.aaper.com/>).
- Storage of samples. Any small vial will work, as long as they do not leak. One fairly inexpensive option is the 1.6ml microcentrifuge tubes obtained from Continental Laboratory Products. A bag of 500 tubes cost \$13.26 from the University of Arizona Stores. (http://www.clpdirect.com/clpdirect/product_item.aspx?prodid=MICROTUBES1)