### Walter Reed Biosystematics Unit Smithsonian Institution Walter Reed Army Institute of Research

# Some Tips on Preservation of Mosquitoes

The purpose of this sheet is to provide you with some basic guidelines on how you can obtain and store the best possible specimens of mosquitoes from your area. Once you have a collection of common species, you will find many uses for it, including school demonstrations, training technicians, and reference. People are usually surprised to learn that more than one kind of mosquito exists and they are impressed by an abatement district that targets its activities on its worst species.

#### Larvae

Larvae collected in the field are best transported alive to the office. The 'whirlpack' plastic sampling bag provides a means of getting the larvae back with a minimum of mortality and damage. these bags are filled about half-way with larvae and water, sealed with the attached twist-ties to form a tight 'pillow', and transported in a thermos or cooler containing a small amount of ice or cool water. Once back at the office, the larger fourthinstar larvae can be preserved immediately by dropping them in hot (not boiling) water. The dead larvae should be preserved in 80% ethyl (available as 'completely denatured alcohol' which is 95%, or sometimes labeled as '70% Ethyl Alcohol', which will do in a pinch) alcohol. The more commonly available isopropyl alcohol will preserve the larvae, but makes them brittle in a few weeks. In placing the larvae in the alcohol, it is best to handle them with a lifter. Though less convenient than a dropper, a lifter avoids damage to hairs and also introduction of water to the alcohol solution. The number of larvae in a vial must be limited in order to prevent gradual decomposition of the specimens. A good rule of thumb is that the larvae should take up no more than 10% of the volume of alcohol solution. For most purposes at an abatement district, preservation of whole larvae in vials of alcohol is probably more appropriate than mounting on slides, since technicians always sort freshly killed, whole material. Small larvae can be reared to fourth instar by placing them in clean, non-chlorinated water (dechlorinated tap water is ideal, but if you use distilled water, be sure to add some organic material like dry oak leaves which will buffer the acidity of the pure water) and providing food in the form of ground fish food (Tetramin E is a convenient source since it is pre-ground).

#### Adults

Handling of adults is very important in getting good specimens. The ideal technique is to rear adults from larvae or pupae, let the mosquitoes develop their cuticle for 24 hours, then kill them. This method produces nearly-perfect specimens and also provides the option of preserving larval and pupal skins individually associated with an adult. Small light traps can provide good specimens if large insects are screened out and a live-bag, rather than a kill jar is used. Care must be taken to avoid collapsing the net bag on the

specimens during transport and preparation because even light pressure will rub off scales and hairs. Other kinds of collections (e.g., landing collections, resting site collections) usually produce good specimens. Killing the mosquitoes can be a problem when they have been collected in numbers. Any of a number of solvents work, including chloroform, carbon tetrachloride, ethyl acetate, etc. Freezing is also effective, but moisture condensation can ruin specimens. Once the mosquitoes are dead, they should be placed in a small paper-covered dish for pointing. Handling of dead mosquitoes should be done by slipping the forceps under the specimen, somewhat in the manner of sliding a fork under food. This avoids damage from pinching the specimen. If you must pinch, do so as lightly as possible on the edge of the wing or on a femur. Pointing is best done under a microscope in order to be sure the mosquito is positioned properly. the point is first manipulated to the top of the pin by grasping the shaft of the pin with a forceps and pushing the point. The point must not be grasped directly because it will loosen from the pin, creating a spinning specimen. Next, the dead mosquito is placed on its left side or its back. The pin with its point at the top is picked up with your fingers and a minute amount of ambroid glue (Elmer's will work, but it is harder to use and not as permanent; we buy ambroid, let it dry, grind it and redissolve it in amyl acetate) placed at the top of the tip of the point by sliding the point along a rod coated with glue. This point is then held against the right side of the thorax of the mosquito, preferably between the second and third legs. The legs are oriented toward the pin so that they do not stick out into space, asking to be broken. Gentle pressure for just a few seconds is usually sufficient to fix the mosquito to the point. The final step is to lower the pint with its affixed specimen on the pin to a level that protects the mosquito from fingters when the pin is picked up by its head. Such specimens can be stored in insect boxes with naphthalene or paradichlorobenzene.

## Sources of Supply

Ambroid Glue: Ambroid Company, P.O. Box 164, Lynnfield, MA 01940, (617) 246-1180. This glue is available in many hobby shops.

Whirlpack Bags: Nasco Sampling Equipment, 1524 Princeton Ave., Modesto, CA 95352, (209) 529-6957 or free phone order service at (800) 558-9595

General collection supplies: BioQuip Products, P.O. Box 61, Santa Monica, CA 90406, (213) 322-6636. This company is not cheap, but it has everything needed to store specimens.