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Field Collection of *Nasonia* (Parasitoid Wasp) Using Baits

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Here we describe our standard method for collecting *Nasonia* using baits. The “bait” in this case is liver that has been fed on by *Sarcophaga bullata* larvae, collected after the mature larvae have dispersed and then kept frozen until use (called “flyliver”). This is attractive to the wasps. It is placed in a large (~ 15cm × 15cm) mesh bag that is hung in appropriate collection spots (such as near birds’ nests or under culverts). Within the large mesh bag is a smaller mesh bag containing 4–6 *Sarcophaga* pupae. These retain the wasps, which will go into the bag and begin stinging the hosts. This smaller bag is placed abutting the flyliver. The mesh bags can be made with standard nylon window screening, or any other material with mesh width large enough to permit entry of the wasps.

MATERIALS

REAGENTS

Flyliver - liver remains fed upon by fly maggots (such as *Sarcophaga*), collected after the maggots have left the liver (From Protocol 2, Rearing *Sarcophaga bullata* fly hosts). They contain a volatile substance that is very attractive to the wasps. The flyliver is kept in a freezer (–20 or –80) for storage until approaching the time of use. For transport, we place the flyliver in Falcon tubes or other impermeable container, which prevents odor leakage. When you arrive at your site, keep this under refrigeration or in the freezer or cooler. Do not use fresh liver or rotten liver that has not been fed upon by fly larvae. It does not attract wasps.

Sarcophaga bullata (or other blowfly or fleshfly) pupae. Store under refrigeration (4–10 C) and in a cooler away from the sun when in the field. Do not let the hosts get wet! Make sure they are in a container that can “breathe”. Otherwise they will die and be of little use. Before using in the baits, a few hosts should be cracked open to check whether they are alive (not deflated) and not too old (eyes white to slightly pink is preferred – no body coloration).

Resealable plastic bags, small “sandwich bags”: These are to place the host mesh bag in, once it is removed from the bait. Wasps will be within this.

Resealable plastic bags, quart size – These are to place the bait mesh bag in when it is collected.

Collecting tubes. 12 × 75mm polypropylene tubes for collection of wasps.

Cotton plugs

Sealable shipping tubes, such as 2mL screw-cap vials, if you plan on preserving insects in ethanol (as opposed to live collecting) for shipment.

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95% ethanol (for preserving collected insects). See note below about air travel with ethanol.

EQUIPMENT

Large mesh bags – around 20 × 20 cm. Made from nylon/plastic window screening. This bag contains the flyliver attractant and the smaller mesh bag that contains *Sarcophaga bullata* fly pupae. The bag can be simply made by folding the window screening and stapling it on two sides, leaving the top open.

Small mesh bags – around 2 by 3 inches. Host pupae (*Sarcophaga* or other fleshfly or blowfly) will be placed in this bag. It is made in the same way as the large one.

Tape and wire or string (for hanging the bags). The wire used to close plastic bags is well suited for this task

PROCEDURE

A. Setting up the Baits

Put a piece of flyliver in the large mesh bag.

Place 3 to 6 hosts in the small mesh bag.

Place the small mesh bag inside the large mesh bag so that the small bag contacts the flyliver.

Set the bait hanging in an appropriate place. The bait should be in a location protected from rain if there is any chance of rain (e.g. under bridges, eaves of buildings, culvert, underside of branches). If you wish to collect *N. longicornis*, *N. giraulti* or *N. oneida*, place the baits near bird nests (e.g. barnswallow nests under culverts or in buildings, within or near bird boxes, against trees with nest holes). Baits can also be placed near or under dead carcasses that have fly pupae under them, or larvae about to disperse. The bait can be tied or taped to an object. If rain is a concern, you can also put a cup or some protecting structure above the bait.

Collect the baits—Wasps will arrive at the bait sometimes quickly (e.g. within an hour or so) or later (by the next day). There are two basic ways to collect the baits. First, you can remove the smaller host mesh bag, quickly put it into a resealable bag, and replace it with a new one (leaving the bait hanging). This can be repeated over several days. Alternatively (or at the end of your collection), you can remove the bait bag with the host bag within it, placing both together in a resealable bag. Keep the bags with hosts/wasps out of direct sunlight. Transport the material back to where you will be processing it. Screen the bait or host mesh bag for wasps. Place them individually in a polypropylene tube with 1–2 fresh hosts and plug the tube with cotton. Let the wasps sting the hosts for 1+ days. You can then ship the wasps/hosts back to your lab, or transport them with you. The offspring will not emerge for 14+ days. Note, you can also keep the hosts from the host bag and use what emerges. Female wasps collected directly from the field are preferred because they represent a “random” sampling of genotypes from the location. Note, if you do not want live wasps, just place the collected females into sealable tubes with 95% ethanol for transport back to the lab. Also note that restrictions in shipping ethanol on airplanes may require you to decant most of this ethanol before shipping, and restoring it back at the lab.

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