GLOBAL MALAISE TRAP PROGRAM: STANDARD OPERATING PROCEDURES

SAMPLING KIT

Package Contents

- ☐ Standard Operating Procedures
- ☐ Biological Materials Analysis Agreement (BMAA) form
- ☐ Sample Log Sheets
- ☐ 1x Malaise trap kit including assembly instruction sheet
- ☐ 4x 500ml Collection bottles
- ☐ 52x 250ml Sample bottles with external and internal labels
- ☐ 1x Decanting lid
- ☐ 1x Small funnel
- ☐ 3x Nitex filters
- ☐ 3x Rubber bands
- ☐ "Scientific Research Specimens" stickers

Required from Participant

- ☐ 95% Ethanol
- ☐ Ethanol waste container
- ☐ Freezer (preferred) or refrigerator for sample storage
- ☐ Return shipping materials

SAMPLING PROTOCOL

It is critical that we employ standardized operating procedures for the Global Malaise Trap Program (GMP). Our coordinated efforts will ensure specimen preservation for sequence analysis and high data quality, permitting the comparison of sites at a global scale. First and foremost, please ensure the Biological Materials Analysis Agreement (BMAA) form is signed and emailed to ccdbcol@uoguelph.ca before you begin.

1. Locality & Positioning

Deploy the trap at a site which is subject to minimal disturbance and ideally in a habitat's climax vegetation (i.e. placement in a national park or other protected area is preferred). When possible, position the trap at a right angle to an insect flight line, in areas with low undergrowth; forest edges or clearings and elevated sites are recommended.

Consider possibilities of wildlife disturbance and/or human vandalism – try to avoid either scenario as much as possible; the trap may be relocated if consistent issues persist after deployment. Ensure that all proper specimen collecting permissions are obtained (i.e. from local authorities, property owners, etc.).

2. Preparation

Fill one collection bottle almost to the top (~400ml) with 95% ethanol at the time of deployment. Do not substitute with other kinds of alcohol or other preservatives.

Four collection bottles are included in your kit so that they can be swapped out in between bottle changes every week. Ensure that the entire sample is transferred to a smaller sample bottle (see transfer instructions below) and then rinse out the used collection bottle with water and let dry, before re-using it for the next collection.

3. Deployment

After arriving to your field site, assemble the trap securely, according to the Malaise trap instruction sheet. When possible, tie the front and/or back ropes to nearby trees for added support. Also, if available, attach the trap poles to a 6- to 8-foot stake or post at its highest points to protect the trap against falling over from high winds, especially if it is placed in an exposed area. For further assembly instructions visit: https://www.youtube.com/watch?v=sU9rW71f5ZA&t=9s

Tightly affix the prepared collection bottle to the trap head; tie the white ropes on the trap around the bottle to secure it. Begin the collection on a day of the week you can consistently return to for the duration of the sampling period.

4. Collection & Monitoring

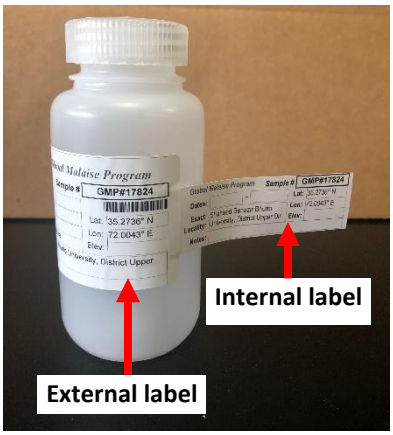
Remove the catch WEEKLY, on the same day each week. Replace the collection bottle with a clean one; refer back to step 2 for instructions. Record collection details in the Sample Log Sheet (examples: trap down at collection, bottle dry, spider web around trap head, etc.).

Visit the trap frequently to monitor for and repair damage, and to avoid sample overflow. In particular, check the trap after strong winds or heavy precipitation. In the event of damage, malfunction or other concerns contact ccdbcol@uoguelph.ca for maintenance suggestions and replacement parts.

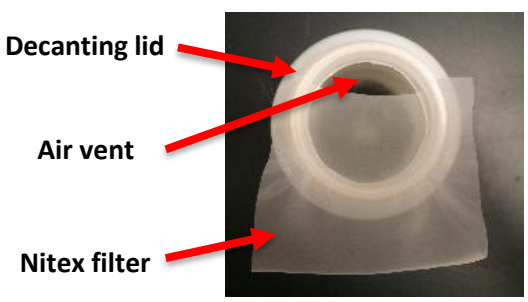
5. Sample Transfer

After changing the collection bottle in the field, sample contents must be transferred into the smaller sample bottles provided for storage and shipping. As much as possible, use the bottles in sequential order based on the number on their lids.

First, fill out the attached external and internal labels with collection details in PENCIL. Then, tear off the internal label and place it inside the bottle.



Using the decanting lid and nitex filter provided, drain off a little more than half (~200ml) of the ethanol from the larger collection bottle leaving enough ethanol that the specimens are still covered. Swirl the sample gently to suspend the samples and then pour the contents into the labelled sample bottle using the funnel provided. Ensure any specimens remaining on the bottle, funnel or nitex filter are rinsed off with ethanol and added to the sample bottle. Thoroughly clean the funnel and nitex filter before using again.



6. Storage

Ideally, place the samples in a standard household freezer (i.e. -20°C) for storage. Ensure that entire insect mass is fully submerged in ethanol before storage; add fresh ethanol to the sample bottle as needed.

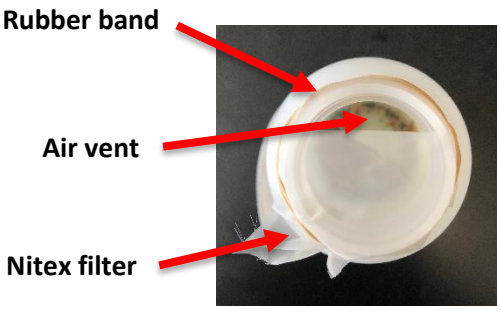
If a freezer is unavailable, store the samples in a refrigerator or cool, dark location. This is critical to preserve the DNA in the samples; improper storage will result in DNA degradation rendering samples unusable for DNA sequencing (e.g. under constant light, heat or variable temperatures).

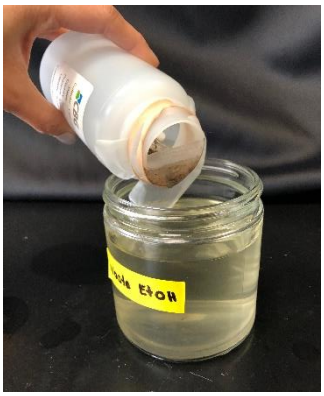
7. Shipment

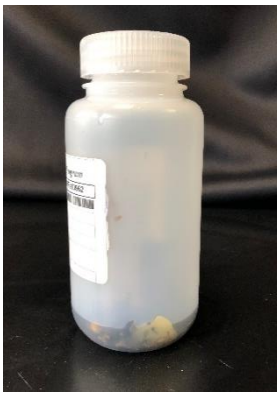
Just before shipment, decant off most of the ethanol from each sample bottle using a nitex filter similar to the sample transfer in step 5. Instead of the decanting lid, use a rubber band to hold the filter around the bottle opening; make sure there is still a small air vent after placement. Thoroughly rinse the nitex filter between samples.

There is a 30ml ethanol maximum per container for shipment and a maximum total of 1L ethanol per package as per International Air Transport Association (IATA) regulations. To follow this protocol, drain the ethanol right to the insect mass. Shipping 26 samples at a time is recommended. Make sure each bottle is tightly sealed and place them into a plastic bag before packaging in a box.

Dispatch samples to the Centre of Biodiversity Genomics, Guelph, Canada using cardboard boxes of appropriate size. Include the completed Sample Log Sheet in your shipment and contact ccdbcol@uoguelph.ca for further shipment instructions.







SHIPMENT CHECKLIST

The first batch of samples should be shipped to CBG after 26 weeks of collecting (or half of the total sampling duration). Use the checklist below to prepare your outgoing package.

- ☐ Notify ccdbcol@uoguelph.ca that you are ready to ship a package; indicate the number of samples you will include and confirm shipping costs
  - Please attach a photo of the Malaise trap and its surrounding habitat to be used for CBG media purposes

- ☐ Return a signed copy of the Biological Materials Analysis Agreement (BMAA) form (e-mail or hard copy)

- ☐ Determine export requirements from country of origin; obtain all proper permits if needed (Canada does not require import documents for scientific specimens)

- ☐ Prepare the samples for shipment immediately before shipping: decant most of the ethanol from each bottle using the materials provided (see instructions above) and close each bottle tightly to avoid leakage

- ☐ Place samples in large plastic bag(s) before packaging them into an appropriate sized box; include the associated sample log sheet(s)

- ☐ Ensure to put a nil (or any permissible minimum) value for customs as the samples have no commercial value

- ☐ For the customs declaration, please write 'Insect Specimens for Scientific Study Only (dead), No Commercial Value'

- ☐ Because the package will contain some ethanol, place the "Scientific Research Specimens" stickers provided on the outside of the package

- ☐ Address the package to the following:

  Sample Submission – Global Malaise Program
  University of Guelph
  Biodiversity Institute of Ontario
  50 Stone Road East
  Guelph, Ontario
  CANADA
  N1G 2W1
  Phone: 519-824-4120 x56393