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Protocol status: Working
We use this protocol and it's working

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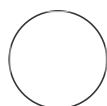
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Collecting needle and branch samples for terpenoid and expression analysis V.3

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ABSTRACT

This protocol is used by the PCG group for sampling needle and branch tissue from hemlock conifers for terpenoid analysis, as well as RNA expression analysis.



IMAGE ATTRIBUTION

The image was taken by Dr. Karl Fetter

TreeSnap Observation


- 1 Open Treesnap (<https://treesnap.org/>), record the accession number on the tree and fill in notes

for hemlock woolly adelgid infestation (HWA) levels (scale provided by the app).

3:41   70%


< Hemlock ?

ADD ENTRY INFORMATION

Images 3 photos added 

Species Eastern hemlock (*Tsuga canadensis*)

What percentage of the branches you see have hemlock Woolly adelgids?

 SHOW EXAMPLES

☐ 0%

☐ 1-24%

☐ 25-49%

☐ 50-74%

☐ 75-100%

☐ I'm not sure

CANCEL

Canopy health 1 - Healthy

Tree diameter 5 cm

Treated	Yes	⌵
Comments	EHS present	
<div>Save</div> <div>Cancel</div>		

Pop-up box displaying the levels of infestation of HWA to record

- 1.1 Use the comment field to enter: *EHS present* OR *EHS absent* to record the hemlock scale.

3:40

<
Hemlock
?

ADD ENTRY
INFORMATION

Woolly adelgids 1 photo added

Cones	No	⌵
Crown classification	Overtopped. This tree's crown is entirely below other trees nearby.	⌵
Habitat	Roadside, urban, suburban, or park	⌵
Trees nearby	Healthy and large	⌵

Canopy health	1 - Healthy	⌵
Tree diameter	5 cm	⌵
Treated	Yes	⌵
Comments	EHS present	
Tree Identifier	335-2011E	
Advanced Options		⌵
Location	42.29700, -71.12577 Accuracy 4 meters More Options	✓
<div> <div>Save</div> <div>Cancel</div> </div>		

highlighting where to place additional notes on individual sampled. EHS is important to log.

- 1.2 Take at least 3 photos with TreeSnap. This includes at least one of the trees itself, one photo of the tree tag if that is present, and one photo of the collection vial with the label as well.
- 1.3 Geolocation will be recorded automatically but TreeSnap can also connect with a more precise external GPS device.
- 1.4 Note DBH in appropriate column

- 1.5 Note any other observation you may have- including plant condition (Excellent/Good/Poor), and placement of individual (lower canopy, full sun, etc.)

Prepare sampling sheet

- 2 The sample sheet would look like this:- [Link to spreadsheet](#)

Collection

- 3 You will collect two sets of samples from each tree. One for RNA extraction for transcriptomics and one for metabolomics. The first needs less material so we will use the 5ml tubes. The latter needs more material and will use the 10ml tubes. The first should include two branches that are approximately 5 cm in length. The second should include four branches, ideally taken from different locations around the tree

Safety information

For RNA collections- please use gloves, and spray RNase before collections (separate vial- 5ml cryovial)

- 4 Metabolomics- You would use 2 10ml vials. You will be able to fit 4 branches (5cm) in each tube- so sample from one direction of the tree, then collect the three other branches from the other sides of the tree (you would ideally try to sample around the individual)
- 5 RNA- You would use one 5ml vial. For this, 2 branches would be more than sufficient to fit into the vial. Ideally, you would want to sample from 2 different locations of the individual.

Safety information

It is important to wear gloves while collecting samples for RNA analysis!

Placing in Liquid Nitrogen

- 6 Close cryovials, and place the vial slowly into the LN dewar. Be wary of LN splashing out, and wear appropriate gear, and stay at a distance.



Safety information

Use appropriate wear while handling liquid nitrogen

Cleaning before sampling from new individual

- 7 Clean trimmers between trees with ethanol (spray bottle).



Note

For the collection of RNA- spray clippers and gloves with RNase and ethanol

- 8 Use a clipboard to manually record notes on a pre-printed spreadsheet with sample names.

- 9 After returning from the field, ensure that notes are consistent with TreeSnap entries. These entries can be amended after collection as needed.

Post-sampling and Pre-shipping

- 10 Remove all vials from the dewar (cross-check with the sample sheet). Samples must be stored at -80C or should be on dry ice during transport.



Cross-check all samples at this stage with your sample sheet.

Shipping

- 11 Shipping instructions will be provided via email. Shipments must be sent on dry ice. You will be

shipping separate parcels for the Metabolomics and RNA samples.

Note

Please separate the RNA and terpenoid vials before shipment

- 12** Log all data and samples in a Google sheet, or send over sample sheet so that we log your data