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

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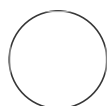
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Collecting needle and branch samples for terpenoid and expression analysis V.2

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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



- 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).

The screenshot shows the Treesnap app interface. At the top, the status bar displays the time 3:41, signal strength, Wi-Fi, and a 70% battery level. The app header is dark green with a back arrow, the title "Hemlock", and a help icon. Below the header are two tabs: "ADD ENTRY" (active) and "INFORMATION". The main content area is a survey form with the following sections:

- Images:** "3 photos added" with a small photo thumbnail.
- Species:** "Eastern hemlock (*Tsuga canadensis*)" with a dropdown arrow.
- Woolly adelgid infestation:** A modal dialog box is open with the question "What percentage of the branches you see have hemlock Woolly adelgids?". It includes a "SHOW EXAMPLES" link and six radio button options: "0%", "1-24%", "25-49%", "50-74%", "75-100%", and "I'm not sure". A "CANCEL" button is at the bottom right of the dialog.
- Canopy health:** "1 - Healthy" with a dropdown arrow.


Tree diameter	5 cm	⌵
Treated	Yes	⌵
Comments	EHS present	
<div>Save</div> <div>Cancel</div>		

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

3:40


70

<
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	✓

Save

Cancel

- 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.)

2 If gathering samples for pooled data, choose four random areas around the tree to collect needle and branch samples

Note

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

3 Look for the second branch from the shoot tip (stick to the right first) and snip off 5 cm to place in 10 ml cryovial

4 Repeat for the other 3 directions (check appropriate column in the sheet provided). Place about 3-4 stems in each 10 ml cryovial.

5 Close cryovial, and using the appropriate gloves to handle liquid nitrogen (LN), place cryovial in LN dewar.

Safety information

Use appropriate wear while handling liquid nitrogen

5.1 Cap vial tightly to prevent opening in the LN.

- 5.2** Depending on dewer configuration, you can tie tubes with string to a 'halo' to help keep vials from falling to the bottom. Fully submerged tubes can open.

Note

You can hold upto 12 cryovials in a 6L dewer

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

Note

For collection for RNA- spray clippers with RNase as well

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

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