



MAR 28, 2023

OPEN ACCESS

Protocol Citation: Vidya S Vuruputoor, Victoria Burton, Jill Wegrzyn 2023. Collecting needle and branch samples for terpenoid and expression analysis. **protocols.io** <https://protocols.io/view/collecting-needle-and-branch-samples-for-terpenoid-crysv7we>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Mar 28, 2023

Last Modified: Mar 28, 2023

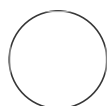
PROTOCOL integer ID:
79602

Collecting needle and branch samples for terpenoid and expression analysis

Vidya S Vuruputoor¹, Victoria Burton¹, Jill Wegrzyn¹

¹UConn

PlantCompGenomics



Vidya S Vuruputoor

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).




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

▼

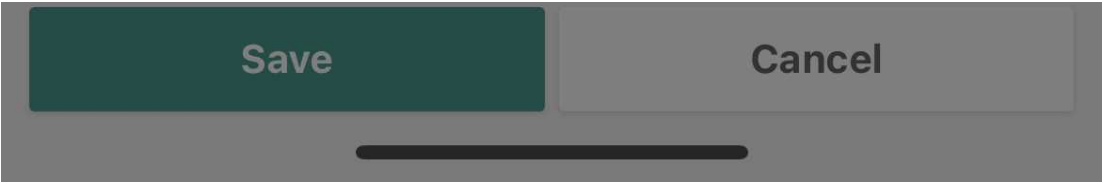
Treated

Yes

▼

Comments

EHS present



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	
<div> <div>Save</div> <div>Cancel</div> </div>		

- 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

- 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 dewar 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 dewar

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