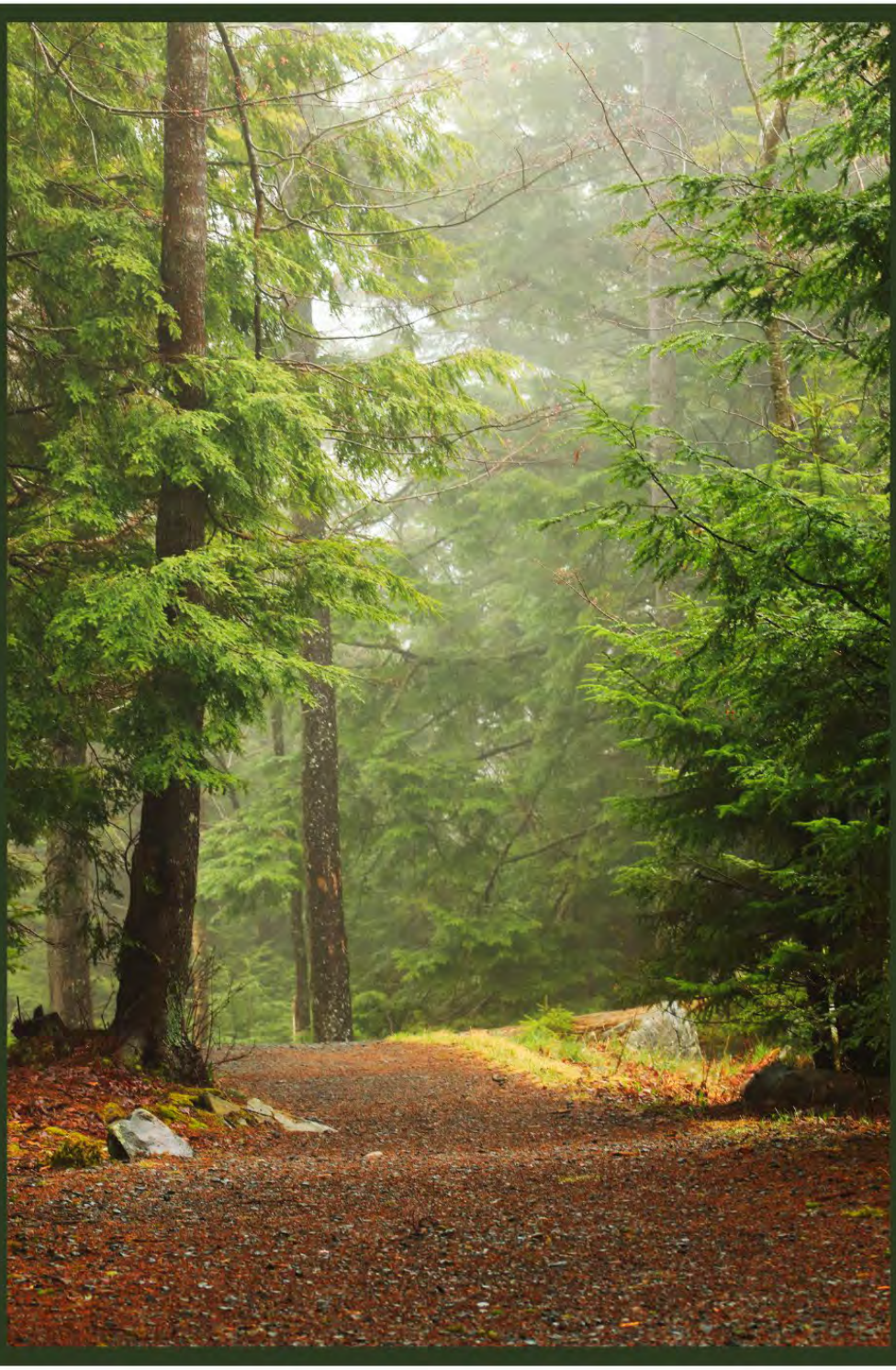
HEMLOCK WOOLLY ADELGID MONITORING NETWORK



2022 SAMPLING PROTOCOL AND INSTRUCTION MANUAL

ISC | NRCan | CFIA

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Acknowledgements

This protocol was developed in partnership by the Invasive Species Centre (ISC) and Natural Resources Canada (NRCan) based on Grand Valley State University's trapping method and Canadian Food Inspection Agency's (CFIA) visual sampling method. Credit must be given to all four organizations for any use or distribution of this protocol.

Funding for this project has been provided by the Canadian Food Inspection Agency and Natural Resources Canada.

We would like to take this opportunity to thank Grand Valley State University for providing expertise and consultation on this program.

We extend a special thanks to the volunteers participating in this exciting program. Volunteer involvement in monitoring for invasive species is appreciated and fundamental to the success of invasive species management efforts. Your support is essential to the success of this program and is very much appreciated. Thank you.

Plant Protection Act

This project is run n collaboration with the Canadian Food Inspection Agency (CFIA). Under the Plant Protection Act, any positive finding of HWA, a species of quarantine significance, may be followed up by the CFIA. By participating in the HWA Monitoring Network you acknowledge that should a species of quarantine significance be found, CFIA has the authority to contact you and/or visit the site for inspection.

Welcome to the Hemlock Woolly Adelgid Monitoring Network

The Hemlock Woolly Adelgid (HWA) Monitoring Network was initiated in 2022 to increase early detection of HWA outside of known distribution and regulated areas. This program is coordinated by Invasive Species Centre, in partnership with Natural Resources Canada and the Canadian Food Inspection Agency.

The HWA Monitoring Network will be an invaluable source of information on the spread and distribution of hemlock woolly adelgid in Ontario. The program offers a unique opportunity for community members to become actively involved in monitoring and stewardship of their woodlots. Successful control of the spread of invasive species is dependent on the most accurate knowledge of distribution of invasive species possible.

Ontario has ***over 400 exotic species***, of which 14 are significant forest pests and diseases. There are new species reported every year adding to the growing list of invasive species. Many of these invaders have already been found to have profound impacts on forest ecosystems. Invasive forest pests are often introduced to North America via live plants, wood packaging and by affixing themselves to transportation vessels. Once they arrive, they can spread rapidly, posing a serious threat to native tree species and the animals that depend on them. Unfortunately, once established, eradication may not be feasible and management strategies can shift from eradication to long term control.

Many negative ecological, economic, and recreational impacts have been observed in North America due to the introduction of invasive species. The importance of preventing the spread of these species cannot be stressed enough. The active participation of everyone, from community members, to contractors, NGOs and business professionals, as well as government, is vital to preventing the spread of these invaders. Our efforts today will benefit the forest ecosystems of tomorrow as well as contribute to our continued enjoyment of the benefits it provides.

What is Hemlock Woolly Adelgid?

Hemlock woolly adelgid (HWA) is an aphid-like insect (aphids suck fluid from plants) that attacks and kills hemlock trees by feeding on nutrient and water storage cells at the base of needles. Their most identifiable feature is the white woolly ovisacs which give them their name and that look like cotton balls or clumps of snow. HWA can spread naturally by wind and animals, and through human movement of nursery stock, logs, and other wood products including firewood.

Researchers believe HWA was first brought to the North America via infested nursery stock from Japan. The pest was first discovered near Richmond, Virginia in the 1950s and has since established along the eastern coast of the United States. In Canada, HWA can now be found across British Columbia, the South-Western half of Nova Scotia and in some areas in Ontario. In Ontario, HWA was initially detected and subsequently eradicated in Etobicoke in 2012 and Niagara Falls in 2013. In 2019, HWA was confirmed in two small populations near Wainfleet and again in Niagara Falls. HWA was also confirmed in Fort Erie in 2021 and more recently in Grafton and Pelham in 2022.

HWA is federally regulated by the Canadian Food Inspection Agency (CFIA), who continues to monitor current populations.

What is eDNA monitoring?

Environmental DNA (eDNA) monitoring is a method of sampling to detect genetic material shed by organisms into the environment. Genetic material can be detected from environmental samples such as soil, water, or snow, where cells, tissue and excrement amongst other sources are expelled by organisms into their surroundings. Using eDNA grants us the opportunity to get the jump on invading species because it can support detection early in the invasion process from even small amounts of genetic material rather than needing to detect a physical organism. However, it does not tell us the number of organisms or whether that organism is currently present in the area or how long it may have been present. For example, the organism may have since passed through or died.

To learn more about eDNA, check out the ‘Enabling Early Detection’ Fact Sheet in Appendix C.

Program Objectives:

The main objectives of the HWA Monitoring Program are to:

- Increase local awareness of HWA and other forest invasive pests
- Encourage greater public involvement in monitoring for HWA, and preventing the spread of HWA and other forest pests
- Establish a volunteer network to track the spread of HWA in Ontario
- Increase monitoring on the landscape by informing and complimenting existing government monitoring programs
- Increase early detection of HWA and Ontario distribution maps such as EDDMapS, the Early Detection and Distribution Mapping System (www.eddmaps.org)

The success of this project depends on the participation of volunteers who will set out eDNA microscope slide traps and visually assess for HWA in their woodlots. The eDNA traps can detect small amounts of DNA, regardless of finding the physical insect while visual assessment will detect the physical presence of the insect, but works best with populations that have been established long enough to be visible from the ground. Combining these two methods will increase the chances of detecting new populations before they have a chance to do damage and spread. The site for this project will be located in high-risk areas to comprehensively monitor leading edges of HWA and suspected movement on the landscape which will also compliment and support current surveillance efforts for HWA by federal and provincial agencies.

Trap distribution will occur in April with active monitoring to occur late April through June when HWA is most mobile and visible on the landscape. Complete instructions on how to deploy traps, and visually assess for HWA as well as returning samples for analysis are described in detail in this manual. Please follow all the steps listed below to ensure that samples and data are collected properly.

This sampling activity requires a minimum of two site visits (set up and take down), although it is recommended to return to the site weekly to ensure the trap is still in place and check for damage. Please read all instructions carefully before heading into the forest.

Note: All volunteers participating in this project are required to have read and signed the volunteer participation waiver before commencing these activities.

Trap Set up and Deployment

1. Please ensure you have appropriate transportation to and from the sampling location and any personal protective equipment and safety equipment appropriate for the site. It is encouraged to travel and work in pairs as an added safety measure.

2. Pick a fair-weather day to conduct sampling (please avoid strong winds/storms)

3. Deploy traps promptly upon receiving to ensure traps are in position for a minimum of two months. The recommended time frame is to have traps in place from April 24th to June 24th 2023. **All Samples MUST be removed from the field and mailed back by June 30th, 2023.**

4. Ensure you have all required trap items (checklist provided) before heading out.

    - You are responsible for obtaining something to hold the trap. This can be a dowel, stake, or pipe that you will push into the ground to a depth of 50 cm. The length of whatever you use must be long enough to hold the trap above ground anywhere from 50 cm (knee height) to 150 cm (chest height).

5. Please inform the ISC of any delays or problems you encounter.

If you have any questions, please email Derissa Vincentini, Project Coordinator at dvincentini@invasivespeciescentre.ca.

Monitoring Kit Materials

Please use this checklist to ensure you received all components of your kit.

- ❑ 1 eDNA trap (one base, one lid, one chain and one tag)
- ❑ 2 50ml tubes containing 2 microscope slides each (4 slides total)
    - o Microscope slides are dipped in petroleum jelly
- ❑ 4 latex free gloves
- ❑ 1 wing nut
- ❑ 1 bolt
- ❑ 1 protocol
- ❑ 1 datasheet
- ❑ 1 return envelope
- ❑ 1 square of corrugated plastic
- ❑ One stake, post, pipe or other stand for trap ***not included***

The following chart breaks down which items get returned to the ISC, which can be kept for the following year (and if not returned to the ISC) and which items can be disposed of at the end.

Stored for next year	Mailed back	Disposable
– 1 eDNA trap (base, lid, chain & tag) – 1 wing nut – 1 bolt – Square of corrugated plastic	– 2-50ml tubes containing 2 microscope slides ea. (4 slides total) – 1 datasheet – 1 return envelope	– 4 latex free gloves – 1 protocol

Please contact Derissa at dvincentini@invasivespeciescentre.ca if anything is missing from your kit.

Step 1: At Home: Inspect and Prepare the Equipment

1. Inspect all equipment to ensure it is intact and accounted for.
2. Ensure the selected stake will support your trap securely and parallel to the sky. Test your set up with corrugated plastic, bolt and wing nut. ***Do not open the trap at this time.***
3. Download the free ArcGIS Survey 123 app to your smartphone or tablet. Once downloaded, scan the following QR Code or click the link to open the Survey 123 form. You will not need an account to be able to access this form and the form will be accessible on the app home screen after first use. https://arcg.is/1jLKbO0

Step 2: Select Your Trap Location

Traps should be placed either in the centre of the hemlock stand, or if the woodlot is large (greater than 50 acres) and hemlock spread evenly throughout, place the trap 200-300m from the edge of the stand. The trap should be placed directly under the crown of a hemlock tree, preferably in an area where there are multiple hemlocks.

Step 3 – Fill Out Field Sheets and Labels

Before heading out, take a few minutes to fill out as much of your field sheets as possible. Please use pencil. Do not use ink, as it will smear if the sheet gets wet. Please provide as complete and accurate information as you are able to, as this will aid any future data analysis.

Tip: Instructions on how to find your GPS coordinates on a smart phone or computer are listed at the end of this booklet under Appendix A.

Step 4 – Travel to your Sample Site

Don't forget to take photos of the site! Photos can include your trap set up, sampling Survey 123, of your volunteer(s) and of the sampling location and conditions.
If you wish to share, submit your photos to www.surveymonkey.com/r/26PKT9T.

Step 5 – Trap Deployment and Set Up

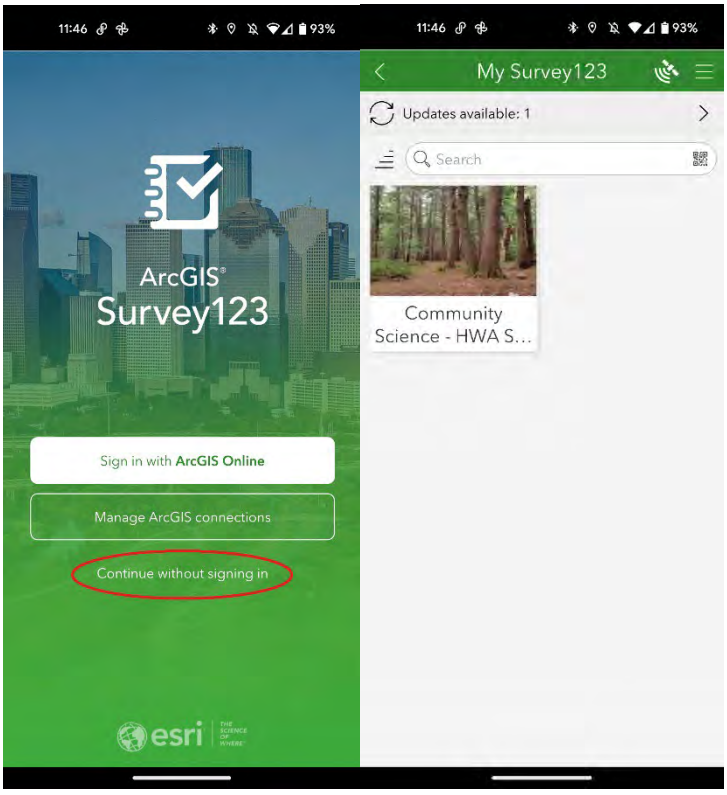
1) Push stake/stand into the ground to hold trap 50-150 cm above the ground and not at risk of touching the foliage of the tree.
2) Put on the provided gloves then open the trap equipment.
3) Assemble your trap following the directions below - carefully open one of the 50 ml tubes and remove one pair of microscope slides:
    a. Do not touch the lower half of the slides covered in petroleum jelly
    b. Separate the slides, noting that the slides are shipped back-to-back with the petroleum jelly facing outwards.
    c. Place one slide in each of the slots on the trap with the petroleum jelly side facing UP and orientated toward the middle of the trap
    d. Replace the lid on the tube. Retain the tube to return slides
4) Repeat 4) with the other two slides in the other 50 ml tube
5) Click the trap lid onto the base and secure with the provided zip tie.
6) Secure the trap to the stake.

It is recommended that you check the trap is undamaged and standing throughout the season

Step 6 – Visual Assessment with Survey 123

Visually survey as many hemlock trees as possible over the two-month period of trap deployment. Ideally, for small woodlots, visually survey all hemlocks to a maximum of 40 trees. For larger areas, aim to survey up to 100 hemlock trees, ensuring they are evenly distributed throughout the forest stand. Stop if these thresholds are met. For the full Survey 123 Protocol, see Appendix B.

1) Open your Survey 123. The form you opened in Step 1 should be on the App home screen. If not, go back to Step 1 to find the link or QR Code.



2) Fill out the Date and Surveyor Information

3) Ensure the GPS Survey Location is accurate

4) Proceed to inspect hemlock trees in your stand

    a. Select Hemlock trees that have branches within easy reach from the ground. It is not necessary to use a ladder or pole pruner.

Note: In mature stands where trees have no low branches, using a pole pruner, periodically remove two branches from the mid-upper canopy of selected trees particularly in high-risk areas. Alternatively use binoculars as light permits or use the ball sampling technique as outlined in the CFS Technical Note 116 (Appendix C).

b. Select a branch with green foliage (needles).

c. Scan the underside of that branch for any evidence of ovisacs, and nymphs focusing your examination along the stem on the outer 1 m of the branch

d. If no woolly masses or nymphs are found on the first branch, select a second branch on the opposite side of the same tree and examine it in the same way.

e. Examine the lower portion of the bole (trunk) for woolly masses or evidence of HWA.

5) While walking between selected trees, look on the ground for hemlock branches that may have broken off from the crown of trees. All shoots should be examined for signs and symptoms of HWA.

6) When walking along roads or open trails with lots of sunlight, use binoculars to periodically scan host trees for signs and symptoms of HWA.

7) Mark any trees that are suspected to have HWA and if possible, take photos of your suspected signs and symptoms. Option to follow-up with branch sampling to obtain an insect sample for lab confirmation (See full protocol in Appendix B).

8) Take pictures of your trees.

9) Record the number of trees inspected in Survey 123 and whether there were any signs and symptoms of HWA.

10) Add in your pictures, roughly draw in the area surveyed and add any additional notes you may have.

11) Submit the Survey 123 Form.

Step 7 – Trap Take Down

1) Traps should be removed around June 24th using the following steps: If you have more than one trap ensure the slides are being placed into the same tubes that came with the trap when the kit was shipped to you.

2) Put on the provided gloves and carefully remove the lid of the trap.

3) Remove 2 slides, place them back-to-back with the petroleum jelly side out and insert into one of the 50 ml tubes, replace the lid of the tube and tighten.

4) Repeat step 2 for the remaining slides.

5) Remove the trap from the stand/stake and pull the stand/stake out of the ground.

Step 8 – Return Shipping

1) Place the two 50 ml slide tubes and datasheet into the provided bubble envelope. Seal the envelope and take to your nearest Canada Post or place in your nearest outgoing mailbox.

Please retain any delivery information Canada Post provides you with.

Step 9 – Trap Storage

Follow these instructions for proper storage of the trap and equipment for participation in the following year.

Note: If at any point you decide to stop participating in the program please contact us to arrange shipping the trap and equipment back

1) To participate year to year the trap needs to be cleaned, disinfected and stored.
    a. Wash the trap with warm soapy water (dish soap works best). Rinse the trap well before proceeding to the next step.
    b. Prepare a 10% bleach solution to disinfect the trap.
        i. In a well-ventilated area add 1 cup of household bleach to 2.5 liters of tap water in a bucket or other container.
        ii. Submerge the trap in the bleach solution
        iii. Allow trap to soak for 30 minutes
        iv. Rinse well with tap water
        v. Air dry on a clean towel or paper towel
    c. Store trap in the provided zipper top bag OR use new zipper top bag. Store the trap in a cool dry place.

Frequently Asked Questions

How do I get my results and when?

All participants will be sent a report summarizing the results of the 2023 survey sometime in late 2023 or early 2024. The data are also shared with NRCan and CFIA for use in their research and survey activities. Any positive detections of HWA may be subject to follow up inspection by the CFIA (see below). The data will also be shared with EDDMapS, a public data tracking and mapping program for the early detection and distribution of invasive species in Ontario. The locations of the traps will be included in public information.

What Will Happen if a Positive Result is Detected?

HWA is a species of quarantine significance by the CFIA. If your visual survey or eDNA results indicates that HWA may be present at your survey site, the CFIA may contact you or visit the site for a follow-up inspection. This inspection would determine the extent of the infestation. For more information, please see the *Plant Protection Act* or the Legislation and Policy page of the ISC website.

If HWA is identified in an area, that area may be added to the list of regulated zones. This may change the scope of this program and may result in no longer require monitoring of this site. If this occurs, the ISC will contact you to ship and return your trap and equipment.

What Will Happen if Results are Negative?

A negative result is NOT a guarantee that HWA do not exist in your woodlot. A negative result means that neither HWA nor HWA DNA was collected by the trap. You are welcome to continue participating in the program.

When do I return my trap?

You can continue to participate in the program until you wish to no longer participate, or the scope of the program changes and your site no longer requires monitoring. Please store your trap until the following monitoring season as which point the ISC will send you new samples to use on the same trap. Should you wish to stop participating in the program, please contact Derissa Vincentini at dvincentini@invasivespeicescentre.ca to coordinate the return of the trap. We greatly appreciate your help in monitoring for HWA across the landscape.

What can I do to prevent the spread of invasive species?

There are general ways to prevent the spread of invasive species through commonly known pathways.

Firstly, learn how to identify invasive species, especially those in your area or threatening your economic, environmental or social wellbeing. However, even without knowing the specific species, there are things we can do to prevent invaders.

Forest Pests

- Don't move firewood or untreated wood products.
- When travelling, check your vehicle and other hard-surfaced equipment (i.e. trailers) for hitch-hiking insects, or egg masses.

On the Trail

- Brush your boots and equipment to remove mud, debris, plant seeds and invertebrate eggs before accidentally transporting them to new trails.
- Stay on the trail and leash your pet to reduce the risk of picking up invasive seeds and eggs.

On the Water

- Clean, drain and dry your boat. It is now the law in Ontario to visually inspect your boat and boating equipment, remove any debris, mud or aquatic vegetation and drain any standing water before moving it overland to new waterbodies. It is recommended to also dry your boat in the sun for at least one week.
- Learn how to identify your baitfish and buy bait from local, reliable sources. Don't dump your bait in water, instead do so on land at least 30 m away from any waterbody.

E-commerce, Horticulture and Pets

- Purchase native plants for gardens, ponds and aquariums.
- Dispose of invasive plants properly by placing them in garbage bags and leaving them on a hard surface in the sun for at least 2 weeks.
- Learn to identify invasive species that could be for sale.
- Never release pets into the natural environment or waterways and keep aquatic pets away from areas that could experience flooding. Remember, don't let it loose!

Thank you for your participation!

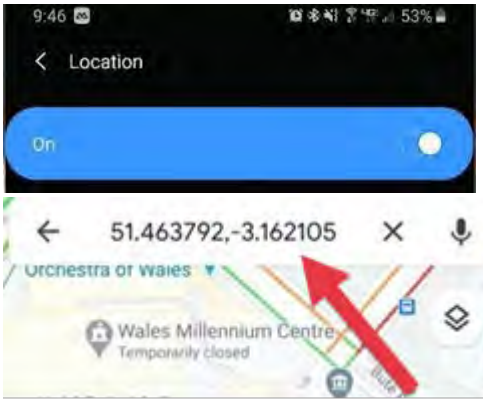
Your participation in the HWA Monitoring Network has increased our knowledge of the distribution of HWA populations across the landscape and would not have been possible without your efforts. The information will be used provincially, nationally and will be added to an international database to track the spread of HWA across North America.

For questions related to the HWA Monitoring Network please contact the Invasive Species Centre:
Derissa Vincentini
dvincentini@invasivespeciescentre.ca
(705) 541-5790

Your participation in the Network is greatly appreciated; we hope you will consider participating again next year if your results are found to be negative. Thank you!

Appendix A: Determine Site Coordinates

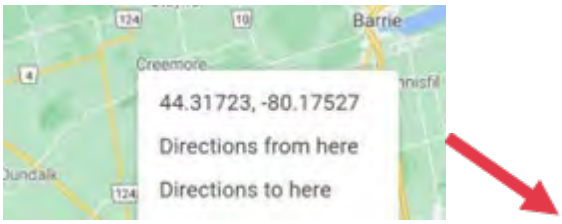
On Google Maps (Android App)



1. Open the Google Maps app.
2. Ensure your location can be accessed by the app. If not:
    i. Swipe down to see your settings.
    ii. Tap ‘Location’ and make sure the switch at the top is on.
3. Drop a pin by touching and holding your current area on the map.
4. You'll see the coordinates in the search box at the top.

On Google Maps (Computer)

- On your computer, open Google Maps.
- Right-click the desired area on the map. You can use the target at the bottom to search your current location.



- Click the latitude and longitude, this will automatically copy the coordinates to your clipboard.

On Google Maps (iPhone or iPad App)

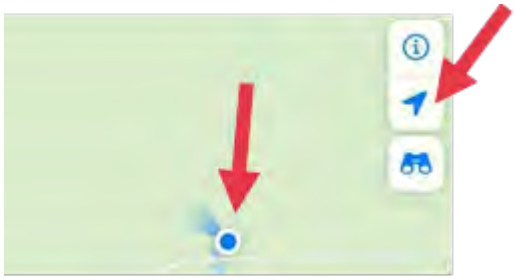
- On your iPhone or iPad, open the Google Maps app.
- Ensure your location can be accessed by

the app. If not:

- Go to your Settings app.
- Scroll down and select ‘Privacy’.
- Make sure the location services are on.

On Maps (iPhone or iPad App)

- Launch the Maps app.
- Ensure your location can be accessed by the app.
  - Go to your Settings app.
  - Scroll down and select ‘Privacy’.
  - Make sure the location services are on.



- Tap the location arrow in the upper right corner of the screen (it will turn blue).
- Tap on the blue dot, which represents your location.
- Swipe up on the information menu to see the coordinates.

Appendix B: Survey 123 Full Protocol Document

HEMLOCK WOOLLY ADELGID

Survey Protocol

AT A GLANCE

- Hemlock woolly adelgid is a tiny sap-sucking insect that attacks and kills hemlock (*Tsuga* spp.)
- Prioritize inspection of host material in urban parks and green spaces, nurseries importing hemlock from infested or regulated areas and hemlock forests within 100 km of known infestations.
- Hemlock trees are inspected for the presence of cottony white egg sacs at the base of needles, twig dieback, discoloured foliage and tree decline

1. Background and Objectives

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand is a destructive pest of susceptible species of hemlock, *Tsuga* spp. and is native to India, Japan, Taiwan, and China. In 1919 HWA was first reported in North America in British Columbia and can now be found in Alaska, Washington, Oregon, Idaho, Montana and California occurring on both western, *T. heterophylla* (Raf.) Sarg. and mountain hemlock, *T. mertensiana* (Bong.). Damage on these two species is usually minor. HWA was first detected in the eastern United States in Virginia in 1951. Since this time it has steadily spread and is now established in Nova Scotia and 20 eastern states and the District of Columbia, where it has resulted in significant mortality of both eastern, *Tsuga canadensis* (L.) Carr. and Carolina , *Tsuga caroliniana* hemlocks. HWA is present in 5 townships in the Niagara region of Ontario, as well as in Grafton, on the north shore of Lake Ontario

This survey supports plant health policy directive D-07-05, *Phytosanitary requirements to prevent the introduction and spread of the hemlock woolly adelgid (Adelges tsugae Annand) from the United States and within Canada*. This visual survey is aimed at early detection of the pest in areas where it is not known to be established.

2. Target Life Stages

HWA nymphs, adults, and white egg sacs through visual surveys.

HWA has a complex life cycle with two successful generations per year in North America (in its native range a third generation attacks spruce). From March through May, the immobile female adelgid (sisten) lays a single white cottony egg sac that contains up to 200 eggs. These egg sacs are deposited on the twigs and can remain on the tree for up to a year. Upon hatching, the nymphs (two types - progredien & sexuparae) crawl in search of a suitable site to settle, usually at the base of a needle where they remain, feed, and develop. The nymphs insert feeding stylets into plant tissue on the underside of the base of the hemlock needle and quickly develop through four instars.

In June and July, the progrediens that are now mature adelgids lay a 2nd generation of white cottony egg sacs on the branches of host trees. Upon hatching, the newly emerged nymphs (sistens), crawl to the base of host needles, feed for a short period and enter a period of inactivity called aestivation from July until October. In October, the young sistens become active, feeding upon the host throughout winter and develop into adults in spring. While feeding the adelgid produces its characteristic woolly covering.

Adelgid feeding at the base of hemlock needles causes needle desiccation (removal of moisture) and the tree tends to take on a grey cast. The resulting needle loss prevents the trees from producing new apical buds and can kill a tree in as little as 4 years. In North America, the sexupara group of adelgids that develop in the spring on hemlock can fly and leave host trees in June in search of a spruce (*Picea* spp.) host. These adelgids have not been successful in attacking spruce species occurring in North America and die without reproducing.

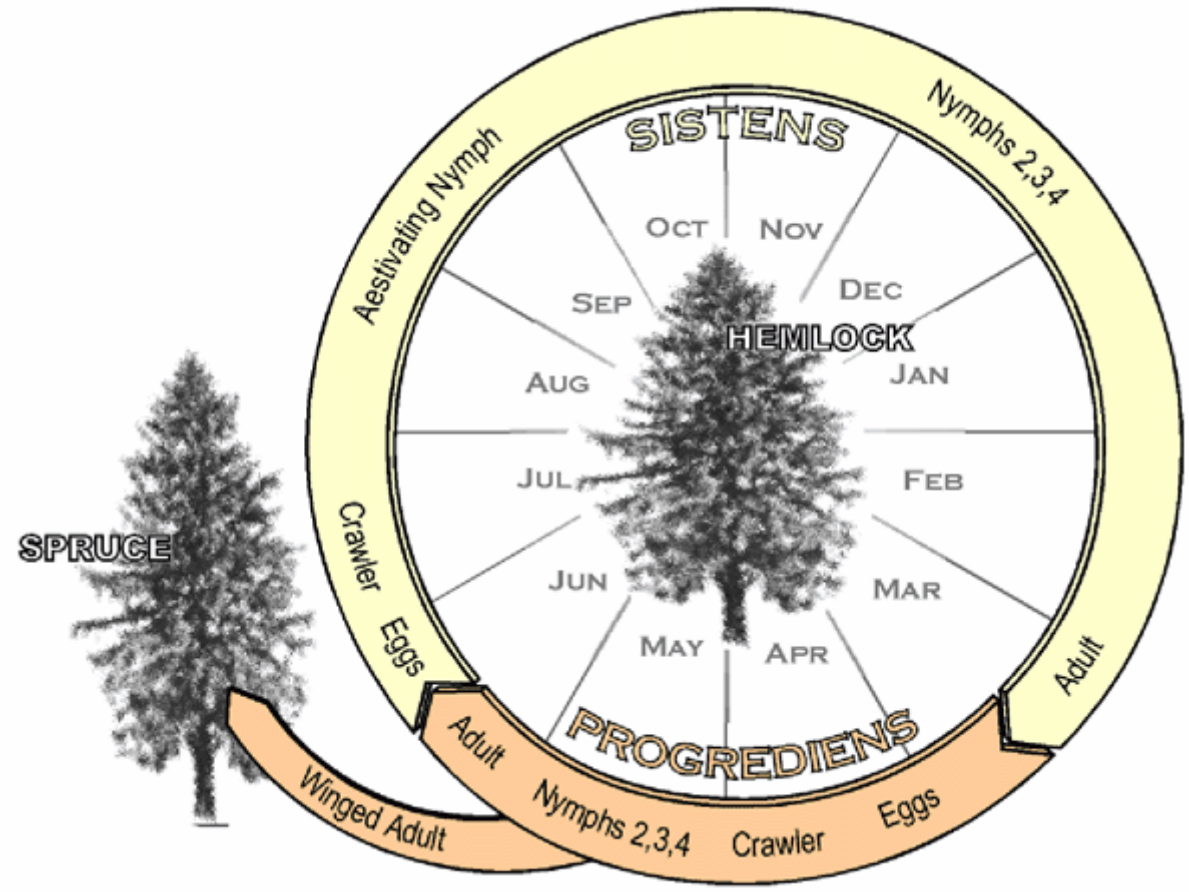


Figure 1. Lifecycle of hemlock woolly adelgid (From Cheah et al. 2004).

3. Target Hosts

All species of hemlock (*Tsuga* spp.*)*

4. Timing and Duration

Biologically, the optimal survey period is from March to May, within an overall survey period running from November to June. **Plan to complete this survey in April and May.**

Visual surveys for HWA are best conducted when obvious woolly masses are present. Although egg sacs can remain on a host tree for up to a year, searching in the summer is not recommended as only remnants of the previous generation's egg masses may be present. If surveys fall outside the recommended time, a hand lens must be used to closely examine shoots for signs of aestivating sistens in summer and fall.

Avoid surveying when snow may be present on the branches of host trees.

5. Target Areas and Site Selection

Given the known distribution of HWA, the primary means by which the pest could be introduced into non-infested areas of Canada is through the movement of infested nursery stock and natural spread by wind, birds, and small mammals. The overall rate of spread has been estimated to be up to 20 km per year in the US however this has been significantly slower in northern areas with an overall average of about 12.5 km/year.

Alabama	Idaho	New Hampshire	Rhode Island
Alaska	Kentucky	New Jersey	South Carolina
California	Maine	New York	Tennessee
Connecticut	Maryland	North Carolina	Vermont
Delaware	Massachusetts	Ohio	Virginia
District of Columbia	Michigan	Oregon	Washington
Georgia	Montana	Pennsylvania	West Virginia

Table 1. List of US states with known hemlock woolly adelgid infestations.

Urban Parks, Green Spaces, and Hemlock Forest Stands

The sole requirement when selecting sites for this survey is the presence of hemlock trees. However, stands of at least 4 hectares and a strong hemlock component are desired. Suitable sites can include natural areas, wooded parks, green belts, and riparian zones. Information regarding hemlock distribution can usually be obtained from provincial ministry of natural resources staff or municipal forest staff. Aerial photographs, forest inventory, and GIS information should be consulted. Wind patterns and migratory routes of birds should be considered in site selection as these both contribute to the spread of HWA. Hemlock forest stands within 100 km of the Canada/US Border should be given priority over other wild sites.

6. Survey Method

The following survey techniques include excerpts and adaptations from the USDA Forest Service publication *Standardizing Sampling for Detection and Monitoring of Hemlock Woolly Adelgid in Eastern Hemlock Forests* (Scott Costa & Bradley Onken, 2006).

Prior to beginning any type of survey, it is important for surveyors to refresh their search image for the most common signs and symptoms of HWA as it may appear in field.

Surveyors should develop a search image for individual or small clusters of woolly masses (Fig. 2) instead of the massive infestations in typical photographs found in many publications.

Figure 2. Individual woolly mass on hemlock twig. (Photo D. Holden)

6.1. Examining the Tree

- Trees are selected simply on the basis of having branches within reach without using a ladder or pole pruner.
- Select a branch with green foliage (needles)
- Scan the underside of that branch for any evidence of ovisacs, and nymphs focusing your examination along the stem on the outer 1 m of the branch
- If no woolly masses or nymphs are found on the first branch, a second branch on the opposite side of the tree is examined.
- Examine the lower portion of the bole for woolly masses or evidence of HWA before proceeding to the next tree (Fig. 3).While walking between selected trees, look on the ground for hemlock branches that may have broken off from the crown of trees. All shoots should be examined for signs and symptoms of HWA (Fig. 4).
- When walking along roads or open trails with lots of sunlight, use binoculars to periodically scan host trees for signs and symptoms of HWA.

Note: in mature stands where trees have no low branches, using a pole pruner, periodically remove two branches from the mid-upper canopy of selected trees particularly in high risk areas. Alternatively use binoculars as light permits or use the ball sampling technique as outlined in the CFS Technical Note 116. (http://publications.gc.ca/collections/collection_2016/rncan-nrcan/Fo123-1-116-eng.pdf)

Mark suspect trees and follow-up with branch sampling to obtain an insect sample for lab confirmation. See section 8.

Figure 3. Evidence of HWA on the bole.
(Photo E. Appleton)

Figure 4. Evidence of HWA on fallen twigs.
(Photo E. Appleton)

6.2. Signs and Symptoms of Attack

6.2.1 Signs of HWA

Ovisacs: The white cottony egg sac is the most obvious stage to detect and can remain on the branches, stems and twigs for up to a year (unless they are rubbed or blown off). Ovisacs occur at the base of the needles and resemble the tips of cotton swabs (Fig. 2). Look along the underside of the terminal and lateral shoots of hemlock branches.

Nymphs and Adults: Nymphs (crawling and sessile) and adults of the hemlock woolly adelgid are very small and difficult to detect. Sessile nymphs are an elongate oval shape, flat, black and might have a slight white fringe around the edges of their bodies (Fig. 5). Use a hand lens to view potential specimens.

Figure 5. HWA nymphs at the base of hemlock needles
(Photo R. Neville)

6.2.2 Symptoms of HWA

Twig Dieback as well as Discoloring of Hemlock Needles: Feeding by HWA results in twig dieback and causes needles to lose their typically green colour. In advanced stages

of the infestation, swollen growths (gouting) occur at the tips of twigs and there is no new formation of branches or twigs. The crown of heavily attacked trees changes in colour from dark to yellowish-green-grey and becomes transparent from needle loss.

6.2.3 Non-HWA Signs and Symptoms

The white woolly masses surrounding each insect are almost invariably located at the base of the hemlock needles. To the untrained observer, other things such as spittle bugs, caterpillar and spider webbing, scale insects, the two white lines on the underside of hemlock needles, and even sap and bird droppings might be mistaken for the adelgid. However, these can be readily distinguished because of their appearance, physical location, or both. Any specimens with questionable features should be bagged and later examined with a microscope or submitted to the Entomology lab for analysis.

6.3. Survey Methodology

6.3.1. Methodology: Urban Parks, Green Spaces, and Hemlock Forest Stands < 3 ha

For small urban parks and greens spaces, inspect all hemlock, to a maximum of 40 trees, or 2 hours per site.

Complete the Survey 123 Site Form (Appendix 1), recording the GPS reading for suspect trees and collect samples as per section 8.1.

6.3.3 Methodology: Urban Parks, Green Spaces, and Hemlock Forest Stands > 3 ha

The objective is to ensure that the trees that are examined are well distributed in the block or stand.

In cases where the hemlocks follow a stream or riparian zone, select a path that follows the edge of the waterbody.

Research has shown that HWA populations have a clumped distribution, as many insects do. This means that infested trees tend to be relatively close together in patches that extend across the forest stand or landscape. As more trees in the stand become infested the clumping diminishes. For the surveyor, this clumping tendency requires that the survey broadly covers the landscape to increase the chance of encountering an infested patch of hemlock trees. This is particularly important when attempting to detect low-level populations. Early infestations are often detected near roadsides or other travel corridors on individual or clumps of trees. An approach that includes both the edges and interior of stands is recommended.

Further research has shown that if no adelgids (woolly ovisacs or nymphs) are found after sampling 100 trees in a hemlock stand (as outlined below) then one can state with 75% reliability that the population of infested trees is less than 2 percent in that area.

- Select a target hemlock stand located within 100 km of the Canada US Border or infested area in Nova Scotia, Ontario, or BC.
- Draw a quick sketch of the site to help you plan your survey approach

- Hemlocks along the edges of waterways should be prioritized and included in the search.
- Obtain a general estimate of the size of the stand. Larger stands can be broken down into blocks that are easier to manage (Fig. 6).
- Record a general assessment of the overall health of the stand. Trees showing decline near the edge of the stand should be examined first.
- Obtain and record a GPS reading for your starting point
- Examine the first tree (see 6.2) for HWA. Take 25 paces (2 steps per pace) along the planned general direction.
- After completing the 25 paces, examine the nearest suitable tree.
- Continue this process using a zig-zagging pattern (Figure 6) until about 100 trees (25 per block) have been examined. Consider the contour of the targeted stand such that your pattern maximizes coverage.
- Complete the Survey 123 Site Form (Appendix 1), record GPS reading for all suspect trees and collect samples (see 8.1).

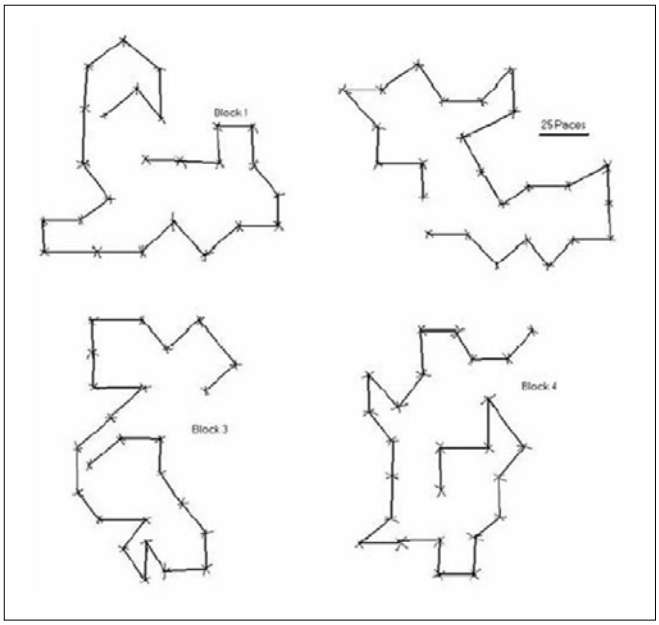


Figure 6. Example of zig-zagging pattern for use in larger hemlock parks, greenspaces, and stands that have been divided into four blocks.

7. Biosecurity Precautions

When visiting areas that are or could be infested with pests of significance, staff must take the necessary precautions to ensure that the risk of spread is mitigated.

- Light coloured clothing is recommended for this survey to make crawlers more apparent.
- Avoid placing gear on or near hemlock trees
- Remove potential crawlers from clothing using a lint roller once you have left the forest stand, before getting into your vehicle.

- Beat and/or lint roll hats and coats
- During the high risk period when crawlers are active (from March to Mid-August), ensure that you do not visit additional survey sites after visiting a positive or potentially positive site.
- Clothing should be laundered prior to visiting another site.
- Vehicles should be parked away from hemlock host trees.
- Clean pruners with a wipe or hand sanitizer prior to leaving each survey site.

8. Sample Handling and Laboratory Submission

8.1 Sampling Procedures

In the event that HWA signs are encountered during the survey, samples should be taken and prepared for submission to the CFIA lab in collaboration with CFIA inspection staff. A digital photograph of the egg sac or symptom should be taken. Contact your local CFIA office http://www.inspection.gc.ca/about-the-cfia/offices/eng/1313255382836/1313256130232 or Area Survey Biologist cfia.surveillance-surveillance.acia@inspection.gc.ca. Suspect twigs or branches should be clipped at least 15 cm below the egg sac using pruning shears and placed in a plastic bag containing a piece of dry paper towel. Record the GPS coordinates in Latitude and Longitude in decimal degrees (NAD 83 datum) for the tree sampled, your name, comments about the site (estimated diameter of tree, health, etc.), the date, and other location information on a piece of paper and place this into the bag. A piece of flagging tape should be placed on the tree sampled as you will likely need to revisit the tree if the sample is positive.

8.2 Collaborative Data Management

Survey activities conducted for a regulated pest in accordance with the established CFIA survey protocol should be captured so that all collaborative efforts can be reported. An Excel spreadsheet containing latitude and longitude coordinates and address for the site surveyed, percent hemlock, organization details and coordinates for any suspect trees can be submitted to the CFIA cfia.surveillance-surveillance.acia@inspection.gc.ca by no later than September 1st each year so that all efforts can be mapped and reported Nationally. Possible suspects should always be reported in real time. Alternatively, data for each site can be entered in a new Survey 123 Site Form (Appendix 1).

9. Supplies

- Knife
- Hand lens
- Digital camera
- Tree identification guide
- Pruning shears
- Re-sealable bags
- HB pencils
- Permanent markers

- Paper labels
- GPS unit & Compass
- Maps (including forest cover or municipal forest inventory)
- Field book with waterproof paper
- Flagging tape
- Blank LSTS Report for Submission forms
- Measuring tape
- Protective footwear (Safety boots)
- Reflective vest
- Sunglasses
- Hat
- Sunscreen
- Lint roller
- Sanitizer
- Pole Pruner
- Binoculars. (Such as 10 x 42) Survey Site Form
- Tick Removal Kit (e.g., https://canlyme.com/product/tick-removal-kit/)

10. Supporting Documents and Additional Information

Pest Fact Sheet for *Adelges tsugae*, Hemlock Woolly Adelgid
http://www.inspection.gc.ca/plants/plant-pests-invasive-species/insects/hemlock-woolly-adelgid/fact-sheet/eng/1325616708296/1325618964954

Hemlock Woolly Adelgid Pest Card

General Information on Plant Health Surveys and *Data Management and LSTS Entry Guidelines for Plant Health Surveys* available on Merlin at:
http://merlin/english/sci/pps/ppse.aspx

Standardizing Sampling for detection and Monitoring of Hemlock Wooly Adelgid in Eastern Hemlock Forests (Scott Costa & Bradley Onken, 2006)
http://www.fs.fed.us/foresthealth/technology/pdfs/HWASampling.pdf

Natural Resources Canada – Canadian Forest Service Frontline Technical Note 116 - *Detection tools for an invasive adelgid*
https://d1ied5g1xfgpx8.cloudfront.net/pdfs/36791.pdf

Hemlock Woolly Adelgid Management Plan For Canada
https://cfs.nrcan.gc.ca/publications?id=39158

Hemlock Woolly Adelgid Fact sheet
https://www.invasivespeciescentre.ca/invasive-species/meet-the-species/invasive-insects/hemlock-woolly-adelgid/

Appendix 1: Survey 123 Guidelines for Hemlock Woolly Adelgid

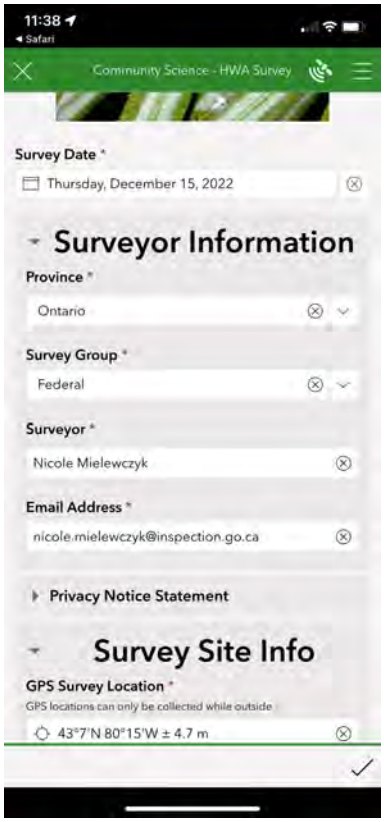
Download the free ArcGIS Survey 123 app to your smartphone. Once downloaded, scan the following QR Code or click the link to open the Survey 123 form. You will not need an account to be able to access this form and the form will be accessible on the app home screen after first use.

https://arcg.is/1jLKbO0

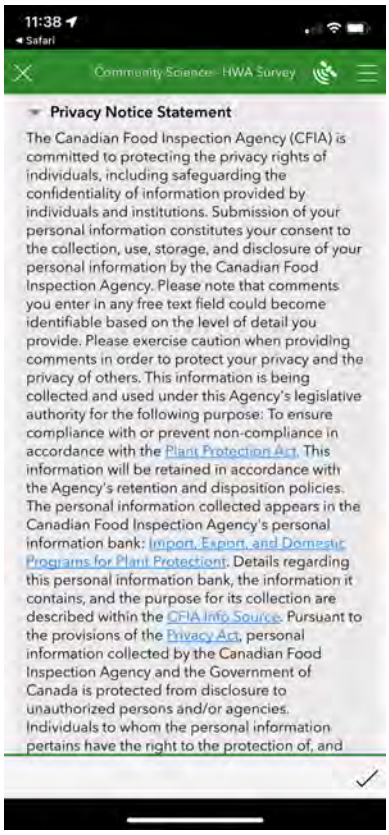
When using your smartphone, select *Open in the Survey 123 field app*.

Browser version can also be used to track survey data, but field app is recommended. All steps remain the same, regardless of the platform used.

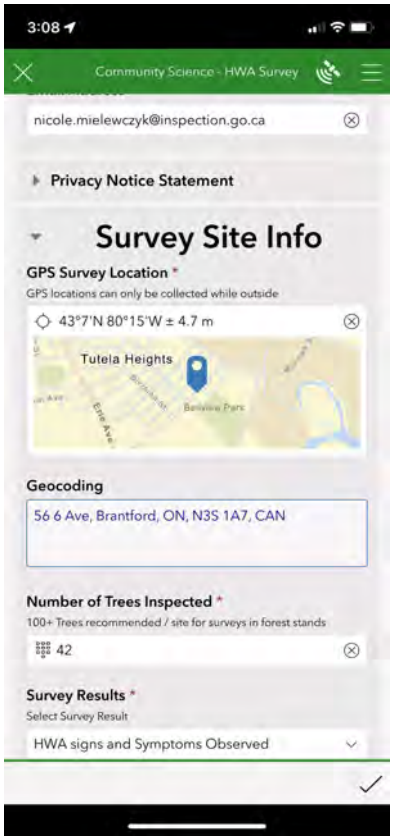
The ***Survey Date*** will be auto populated with the current date. This can be modified and backdated if required.



In the ***Surveyor Information*** section you will capture your information by first selecting the province and group you are part of from the pick list. Then type in your name and email address. These fields are required so that CFIA can contact you to follow up on signs/symptoms of HWA



Select the *Privacy Notice Statement* tab to review details here.

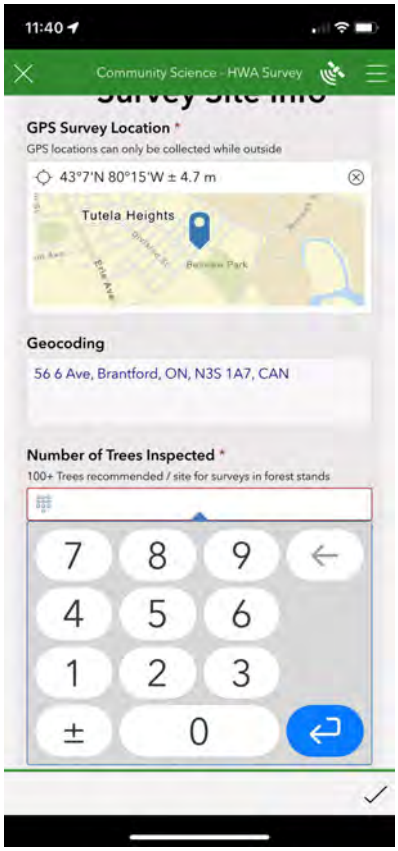


Then proceed to the Survey Site Info section.

In ***GPS Survey Location*** select the crosshairs icon or the map to capture the GPS. This will populate the *Geocoding field* with an address nearby.

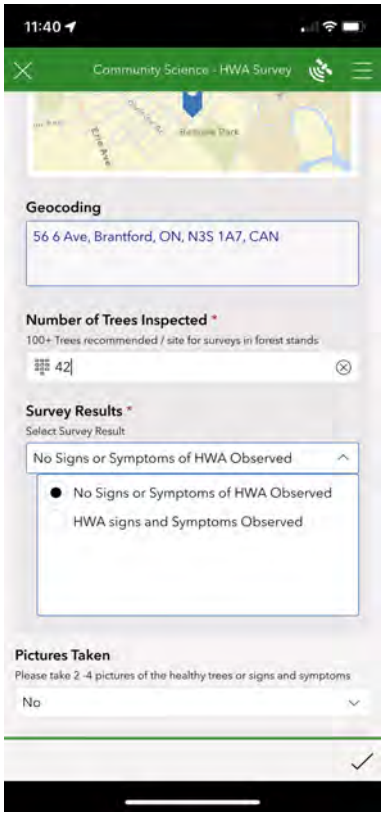
Note: When the GPS reading is plus or minus **more than 10 m** (+- 4.7m in example provided) a more accurate reading should be taken. Press on the map display and a new screen will open allowing a more precise site location to be specified.

Position your location under the cross hairs zooming in as required. Press the check mark in the bottom right when satisfied.

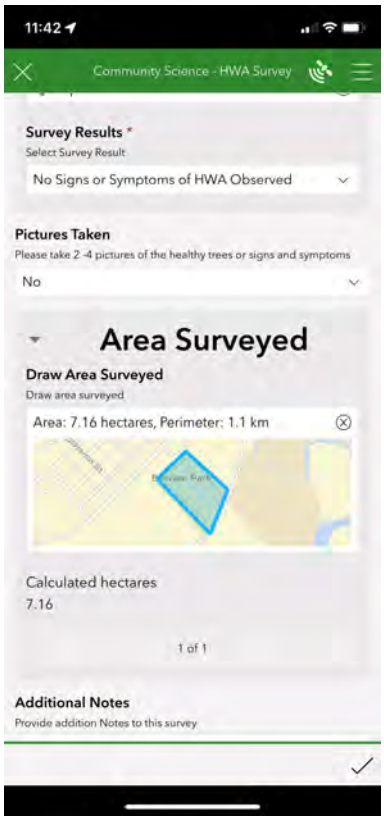


Enter the ***Number of Trees Inspected***. Review Section 6.3 of Survey Protocol to determine optimal number of trees for the size of a site.

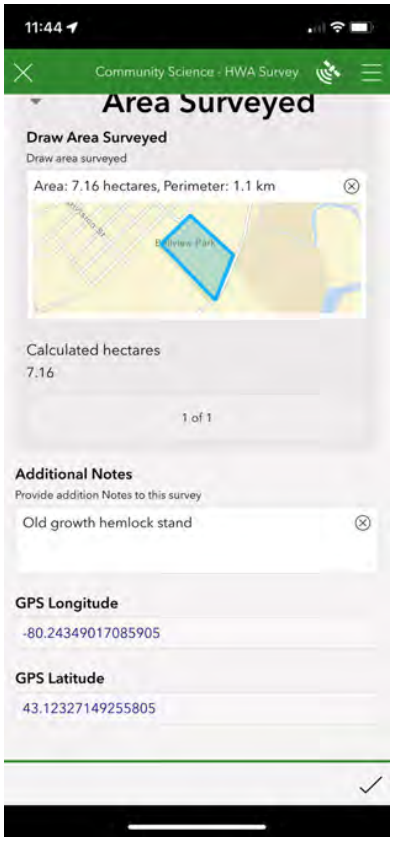
If unable to complete a full formal survey, even a small amount of trees inspected is helpful to support the early detection of HWA



Select **No Signs or Symptoms of HWA Observed** when nothing is found.



Press on the map below ***Area Surveyed*** and a new screen will open allowing a polygon to be created to calculate the area surveyed.



Type in any ***Additional Notes*** and once you're satisfied with the data that's been entered, click the check mark in the bottom right to complete the Visual survey form.

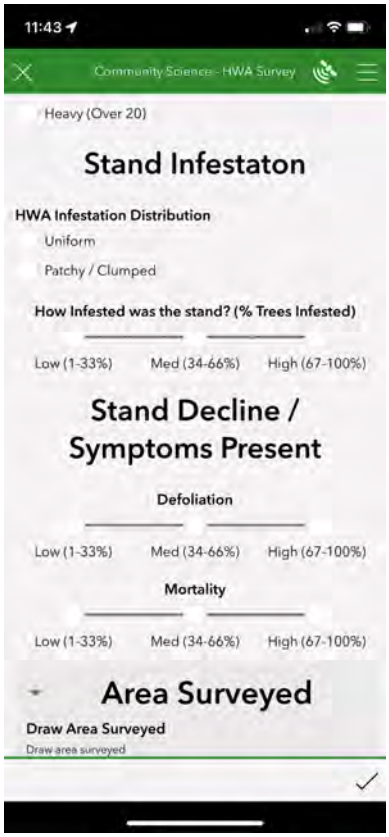
If **HWA Signs or Symptoms Observed** please collect pictures showing the branch, overall tree and then close up shots showing the areas affected.

Note: Contact the Canadian Food Inspection Agency when Signs or Symptoms of HWA are observed.

cfia.surveillance-surveillance.acia@inspection.gc.ca

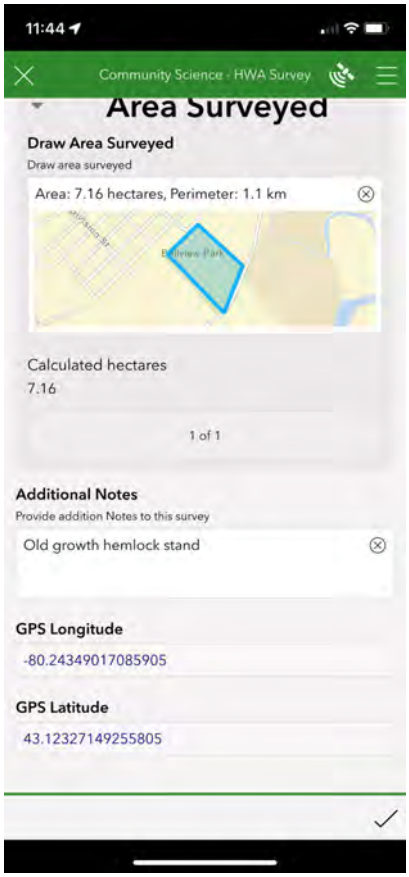
If more than one suspect specimen is identified in distinctly separate areas, submit an additional visual survey form for each specimen collected.

Record the ***Signs Observed*** and average level of ***infestation of each infested tree***.



Record the ***HWA Infestation Distribution*** and the percentage of ***Stand Infestation***.

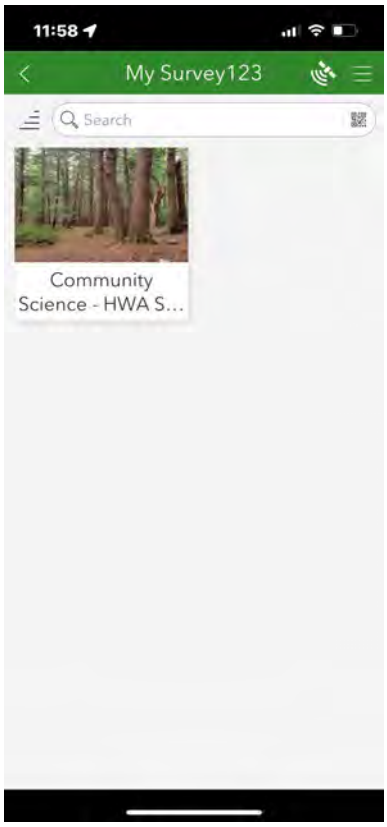
Next record the percentage of ***Stand Decline / Symptoms Present***.



Press on the map below ***Stand Area*** and a new screen will open allowing a polygon to be created to calculate the area surveyed.

Type in any ***Additional Notes*** and once you're satisfied with the data that's been entered, click the check mark in the bottom right to complete visual survey form.

When using the field app for future data collection, select *Continue without signing in*.



The Community Science – HWA Survey form will be accessible on the home screen.

Appendix C: Other Monitoring Protocols

Natural Resources Canada | Ressources naturelles Canada

BULLETIN 89

Frontline EXPRESS

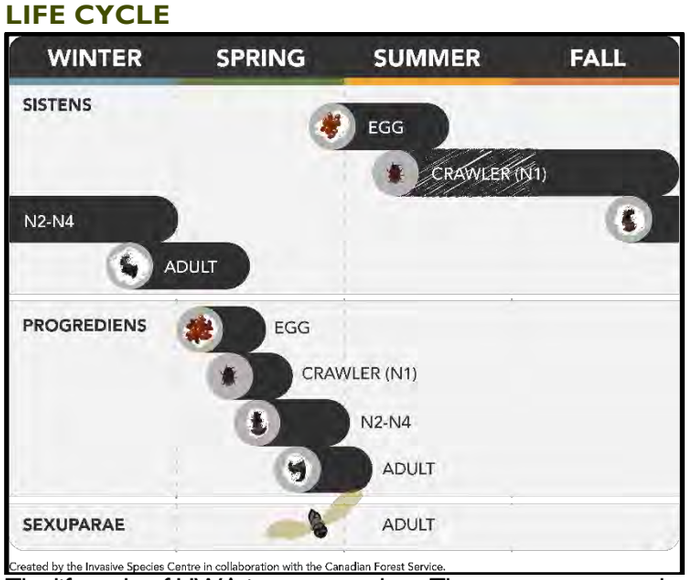
Canadian Forest Service – The Great Lakes Forestry Centre

Hemlock Woolly Adelgid

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsuga*e, is a non-native, invasive aphid-like insect that is attacking and killing eastern hemlock (*Tsuga canadensis*) trees in eastern Canada. The insect is native to east Asia and northwestern North America and was introduced to eastern North America from Japan likely in the 1920s. Since then, the invaded range of HWA has expanded across the northeastern US and in Michigan and Ohio. HWA was detected in eastern Canada in 2012, though populations were likely present for several years prior to their discovery. HWA is a highly damaging pest in eastern North America: up to 95% of the hemlocks in a stand can be killed by HWA over a 3-15 year period. In western Canada, the insect is not a pest and under normal conditions does not kill western hemlock. Hemlock is often found in riparian forests, where it is considered to be an ecological foundation species. The hemlock canopy moderates air temperatures throughout the year, creating a unique ecosystem that provides critical habitat for many organisms. When planted in urban areas, hemlock provides significant ecosystem services and important aesthetic and economic values to humans. Unfortunately, the abundance of hemlock on the landscape has decreased by 60-80% due to land use changes and harvesting. HWA is a major threat to what remains of eastern Canada's hemlock forest and is regulated by the Canadian Food Inspection Agency (CFIA).

LIFE CYCLE



The life cycle of HWA is very complex. There are two asexual generations annually (sistens, progrediens) on eastern hemlock. There is also a winged form (sexuparae) that develops along with the progrediens generation and then migrates to spruce to initiate a sexual generation. However, the sexual generation does not occur in eastern North America.

The sistens generation develops on hemlock from late fall through spring, whereas the progrediens generation develops during late spring and early summer, which we will refer to as winter and spring generations, respectively. Both generations develop under a protective white wool-like covering, called an ovisac. Adults of the winter generation will deposit up to 300 eggs inside their ovisacs that hatch to become the spring generation. These newly hatched 1st instar nymphs (called crawlers, the only mobile stage of HWA) settle at the base of a needle along the twigs. After settling, the crawlers become sessile for the rest of their lives, developing through the remaining three nymphal instars to reach maturity in about a month and a half. Adults of the spring generation will deposit up to 100 winter generation eggs inside their ovisacs. The biology of the winter and spring generations differ in two ways: first, the crawlers settle on new hemlock shoots rather than 1-yr-old twigs; and then after the crawlers have settled, they aestivate, or become dormant, from summer to mid-fall. Once awake, the nymphs feed on the hemlock tree until they mature in early spring. The timing of each generation is climate dependent. For example, in Nova Scotia and Ontario spring generation eggs are deposited in April and May, whereas eggs are deposited in February and March in the southeastern U.S. Dispersal of eggs and crawlers occurs through wind, birds, and other animals at rates of 9- 20 km per year, but spread over much longer distances occurs during spring bird migration and via the movement of infested materials. Only one egg or crawler is needed to initiate a new outbreak.

APPEARANCE

Due to its small size, HWA is most easily recognizable by the white waxy wool masses (ovisacs) it creates near the base of the needles along the underside of the stem. These ovisacs look like the tip of a cotton swab.

HWA white woolly ovisacs on a hemlock twig.

All life stages of HWA are small (< 1.5 mm) and may require the use of a microscope to identify. Eggs are an amber colour and oval shaped. The newly hatched crawlers are football shaped and brown, but once settled they become black with a woolly fringe. The four nymphal instars are almost identical in appearance among the generations and as the insect grows it sheds its outer skin, which remain in the ovisac.

Although unable to persist and successfully reproduce, the winged adults can be used for identifying or surveying HWA. The winged form is distinct from the winter and spring generations due to its wings, longer 5-segmented antennae, and compound eyes.

DAMAGE

HWA feeds on the contents of hemlock's nutrient storing cells and may inject saliva to help digest the nutrient-containing compounds before consumption. To do this it uses its mouthparts, which are more than three times the length of its body, to reach deep in the tree's twigs. This feeding initiates a tree-wide defensive response that can cause a reduction in photosynthesis and causes the tree to show signs associated with water stress (e.g., reduced water movement, and exchange of carbon dioxide and water with the air). Initially, buds die and needles turn yellow. From a distance, heavily

infested trees appear greyish- or yellowish-green. The crown will thin as needles are lost and eventually the tree will likely die, causing stands to look grey. The damaging effects of HWA to eastern hemlock in Canada can be visible 2-4 years after the tree becomes infested, with death occurring in 5-15 years. Eastern hemlocks may die more quickly if they are also affected by drought conditions.

CONTROL

It may be possible to eradicate small infestations or newly-established populations by cutting and burning all infested trees, however, detecting and delimiting small HWA populations is extremely difficult so eradication is rarely a viable management tactic. Thinning of hemlock stands, which increases exposure of HWA to abiotic stress (e.g., increased light, extreme temperatures), can reduce the survival of populations. Even if HWA populations suffer high mortality due to extreme winter temperatures, scientists suspect that the winter generation may actually damage the new shoots when inserting their mouthparts into the vascular tissues, reducing their ability to grow and regenerate.

Horticultural oils and insecticidal soaps can be used to manage HWA infestations on nursery trees. Some systemic insecticides are registered for use in Canada or are being considered for registration.

There are a number of predators and fungi that attack HWA. Extreme fluctuations in winter temperatures can also slow the rate of growth or reduce populations of HWA. Biological control is one tactic that can be used to manage invasive forest pests. Eight species of predatory insects have been introduced to the eastern US from Japan, China, and the northwestern US since 1995, of which 3 species have established populations. The effectiveness of these biological control agents continue to be monitored in the US. No biological control agents have been introduced to eastern Canada, however, scientists believe that predators from western Canada could be promising control agents in the east.

WHAT CAN I DO?

In eastern Canada property owners, forest managers, and commercial nurseries should report all suspected HWA infestations to CFIA and the provincial agency responsible for forest health.

Homeowners can inspect individual trees for signs and symptoms of HWA infestations during spring and early summer when the wool is most apparent. Woolly ovisacs may be present on the bark of the tree's trunk, on twigs, and on branch tips. The ground around hemlock trees can also be checked for fallen twigs and branches with evidence of HWA. Homeowners can minimize the exacerbating effect of drought by making sure trees are well watered. In infested areas, an arborist can be consulted to advise on appropriate management. It is important to remember that when chemical controls are recommended they must be applied by a licenced pest control applicator in accordance with provincial and federal regulations.

Woodlot owners and forest managers can also survey for HWA in the spring and summer. If after visual inspection no wool is found, a recently developed slingshot technique, called ball sampling, is effective at detecting infestations in the upper hemlock canopy. Another detection tool involves the use of sticky traps, but this technique requires a microscope to inspect the traps. In the US, crown dieback of hemlock stands is directly correlated to HWA and can be detected remotely through aerial or satellite imagery. Management tactics for HWA for forest stands are limited, though thinning is one option that may increase HWA mortality.

Commercial nurseries can limit the risk of spreading HWA by only sourcing hemlock from HWA-free areas or purchasing uninfested material. Nursery owners can manage local infestations of HWA by having a licenced applicator apply horticultural oils and insecticidal soaps according to label instructions.

Hyperdog™ ball launcher and Velcro® - covered racquet balls.

SAMPLING TECHNIQUE INSTRUCTIONAL VIDEOS AND FURTHER RESOURCES

Ball Sampling:
https://www.youtube.com/watch?v=0uurPleLOlY&t=2s

Sticky Trapping:
https://www.youtube.com/watch?v=0uurPleLOlY&t=2s

Hemlock Woolly Adelgid - Survey Protocol (CFIA):
https://www.invasivespeciescentre.ca/wp-content/uploads/2021/03/CFIA_ACIA-10300082-v6-adestu_E_External.pdf

Hemlock Woolly Adelgid Management Plan for Canada (NRCan):
https://cfs.nrcan.gc.ca/pubwarehouse/pdfs/39158.pdf

CONTACT INFORMATION

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E-mail: GLFCWeb@nrcan-rncan.gc.ca
Web site: nrcan.gc.ca/forests/research-centres/glfc/13459

Aussi disponible en français sous les titre : ***Nouvelles Express, Bulletin No 89.***

ISSN 1496-7847 (Print) ISSN 1496-7855 (Online)

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Frontline
FORESTRY RESEARCH APPLICATIONS

Canadian Forest Service – The Great Lakes Forestry Centre

Technical Note 118

Sampling techniques to detect canopy infestations of the hemlock woolly adelgid

INTRODUCTION

The Great Lakes Forestry Centre (GLFC) has developed two early-detection sampling techniques: ball sampling and sticky trapping that detect canopy infestations of the non-native hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA). This insect invaded Canada in the provinces of Nova Scotia (2017) and Ontario (2013) and is killing eastern hemlock, an important ecological foundation species. Hemlock stands greatly moderate temperature and intercept precipitation, which allows several wildlife species to flourish during critical times of the year. In order to manage HWA effectively, it is important to detect populations when they are small and before they begin to kill trees. This note describes how these two techniques are used to detect a small or newly established population of HWA in a hemlock stand. We define a small population as one where approximately 2% or more of the twigs have HWA or 2% or more of the hemlock trees in a stand are infested with HWA.

WHERE SHOULD YOU SAMPLE?

Focus sampling along the edges of a hemlock stand, as that is where populations of HWA tend to establish, such as alongside streams, lakes, trails and roads. Also, prioritize trees within 50 metres of the stand's edge and on the windward edges of stands, where HWA has been moved by wind and wildlife.

For both techniques, establish the sampling sites along a haphazard path through the stand, rather than along any one cardinal direction (for an example, see Figure 1). There should be at least a 25 metres spacing between each survey location, and two-thirds of all sampling locations taken within 50 metres of the edge of the stand.

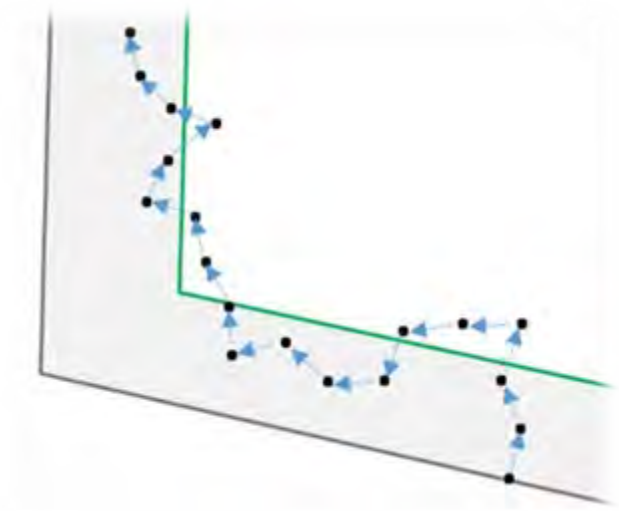

Figure 1. Example of the pattern of sampling using the ball sampling technique along a hemlock stand's edge. Grayed area is the 50 metre buffer from the stand's edge (grey to green lines). Dots represent sampling locations spaced approximately 25 metres apart. At each dot, the nearest hemlock tree is ball sampled. Arrows indicate direction of travel.

WHEN SHOULD YOU SAMPLE?

Ball sampling and sticky trapping should be done between late spring and early summer for maximum effectiveness. Ball sampling can be done at other times of the year, but is less effective.

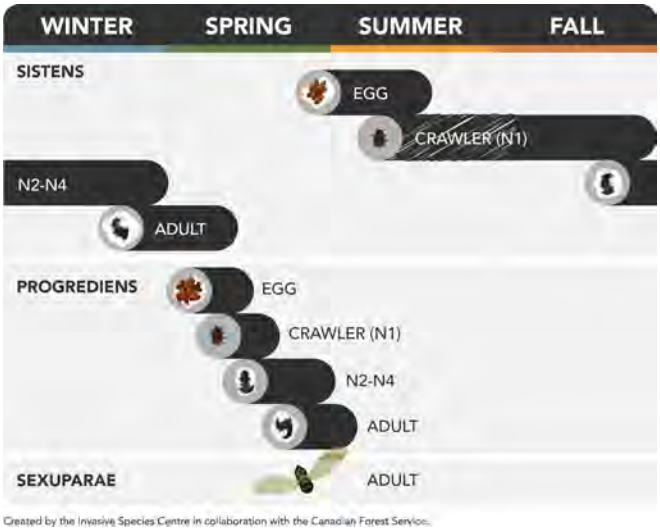

Figure 2. Lifecycle of HWA. There are two generations, three adult forms and three stages of development (egg, nymph and adult).

HOW SHOULD YOU SAMPLE?

The sampling techniques capitalize on two features of the biology of HWA. Ball sampling targets the wool produced by the HWA (Figure 3A), which is present all year on branch tips (Figure 3B). Sticky trapping targets the mobile forms of HWA, called crawlers, which are 1st instar nymphs produced by the two generations of HWA (Figure 3C). These crawlers dislodge from infested trees and fall to the ground. The sticky cards placed under the hemlock canopy intercept the falling nymphs (Figure 3D).

Ball sampling

Construction of the Velcro-covered racquetballs is straightforward and their use is outlined in a video (https://www.youtube.com/watch?v=0uurPleLOIY). GLFC researchers have used this device to sample several hundred trees across a range of HWA infestation levels and developed estimates of the probability of detecting an infested tree for small HWA populations. These estimates have been prepared for sampling both individual trees (Table 1) and hemlock stands (Table 2). Two surveyors can assess a tree by standing opposite each other with the tree crown between them and shooting their ball at a new part of the upper crown for each sample they take. Each ball should contact at least three branch tips as it ascends through the crown. When the ball lands, the person opposite can recover it, assess it for wool, and attempt another sample. Sampling stops when wool is found on a ball or when the desired number of samples have been taken from each tree. Using this technique, an experienced pair can complete **four samples per minute**. A single surveyor can complete a survey using this technique, but it will take considerably more time.

Any samples found on the ball that are suspected to be HWA can be removed and place in 95% ethanol for later identification by an expert.

Caution: This technique involves shooting racquetballs at high velocity into the crown of trees. Sample only when and where the risk to bystanders and property is low. Surveyors using this method should use appropriate personal protective equipment (e.g., helmet, safety glasses). Gloves are highly recommended. We recommend wearing black-coloured leather or suede gloves because white, grey or beige coloured fabric gloves could transfer to the ball, giving a false-impression that HWA wool is present.

Table 1. Recommended number of ball samples to detect a small infestation of HWA in an individual tree, for a range of detection probabilities. Note: probabilities will be higher than listed if the incidence of HWA in the tree exceeds the level for a small HWA infestation that is defined as one where approximately 2% or more of the twigs have HWA or 2% or more of the hemlock trees in a stand are infested with HWA.

To detect an HWA infestation in a tree with a probability of at least:	Number of ball samples required:
35%	5
50%	10
60%	15
70%	20
75%	25
80%	30

Table 2. Recommended number of trees to sample to detect a small HWA infestation in a hemlock stand, for a range of detection probabilities. For a stand-level survey, the recommendation is to take 10 samples per tree. Method assumes a stocking of 2,000 hemlock trees in a four-hectare hemlock stand.

To detect an HWA infestation in a stand with a probability of:	Number of trees to sample:
75%	130
85%	180
95%	270

Sticky trapping

Using sticky traps to survey for HWA is simple and their construction and use is explained in a video (https://www.youtube.com/watch?v=YfGaF7DbODo). The sticky cards are made from rigid corrugated plastic cut to a 20 cm × 20 cm square. GLFC researchers developed this technique using green prism traps sold in Canada to trap the emerald ash borer. These traps come

pre-coated with a sticky film that traps any insect that lands on them. However, any corrugated plastic can be used to construct the traps, as long as one side is coated with a long-lasting adhesive.

One person can set up traps. To set up, place a wooden stake under a hemlock tree, then centre the sticky trap on the stake and nail it in place. Use an array of two traps at each survey location, spaced 30 metres apart, within 50 metres of the stand's edge (Figure 1). Leave the traps in the stand for 5-7 days. Traps can be left out for longer, but they will accumulate by-catch and debris, which makes assessment more difficult. When the traps are collected, place each in its own 3.78 litre resealable zipper freezer bag and store in a freezer until it can be assessed.

When ready to assess, allow the sample to thaw briefly then place it under the objective of a dissecting microscope and systematically inspect it for crawlers (Figure 3C). The assessment is stopped upon discovery of the first crawler.

A single, two-trap array has a 75% detection rate for small HWA infestations. An array takes 15 minutes to set up and approximately 20 minutes to inspect for crawlers. If a third trap is added to the array, detection rate increases to 87% but this adds an additional 15 minutes to the setup and inspection time.

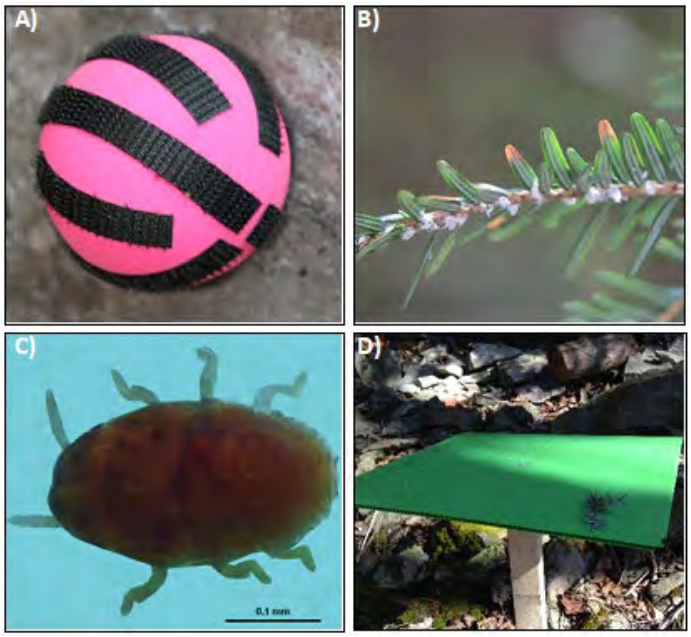

Figure 3. A) Ball sampling; a racquetball used to detect HWA wool that is covered with 50 cm^2 Velcro in 1-cm-wide strips. The ball is shot into the hemlock crown using a slingshot. **B)** wool (i.e., ovisacs) produced by HWA nymphs and adults; **C)** a dislodged HWA crawler (1st instar nymph) caught on sticky trap; **D)** Sticky trapping; a 20 cm-wide × 20 cm-long × 0.4 cm-high The trap is made from green corrugated plastic and is set up about 1.5 m above ground on a 2 cm-wide × 2 cm-long × 200 cm-high wooden stake.

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Appendix D: Resources

The Invasive Species Centre website is a great place to start looking for information and resources on invasive species from both the ISC and its partners, as well as ways to get involved.

Resources:

Community Science Program: Learn how to get involved in initiatives and projects currently happening.

Past Webinars: Our YouTube Channel serves as a platform to share training videos, and informative webinars about invasive species.

Online Training Program: Virtual, self-paced courses for those looking to further their learning in invasive species prevention and management.

Species Profiles: Meet the species that are invasive across Canada; pests, plants, pathogens, fish, animals and invertebrates!

Best Management Practices Database: A collection of easily accessible resources from a wide range of organizations focused on invasive species management recommendations.

Factsheets and Resources: These in-depth invasive species resources include technical bulletins, fact sheets, best management practices, and videos.

Teaching about Invasive Species: See our list of free teaching resources to help teach and learn about invasive species in Canada.

Invasive Species Awareness Week: You can get involved by liking and sharing posts created by participating organizations, or posting with the hashtag #InvSpWk during ISAW.

Green Shovels: Collaborative, shovel-ready projects to achieve job creation, economic recovery and environmental progress on invasive species.

Asian Carp Canada: Your resource for information and news regarding the overall threat of Asian carps to the Great Lakes and beyond.

The Canadian Food Inspection Agency website has information on federal regulations on the movement of goods and current status of invasive forest pests and quarantine zones.

The Government of Canada has an overview of invasive species in Canada on their website.

The Ontario Government has guidance on preventing the spread of invasive species and status of invasive species in Ontario, as well as how Ontario is managing invasive species.