



## Sampling techniques to detect canopy infestations of the hemlock woolly adelgid

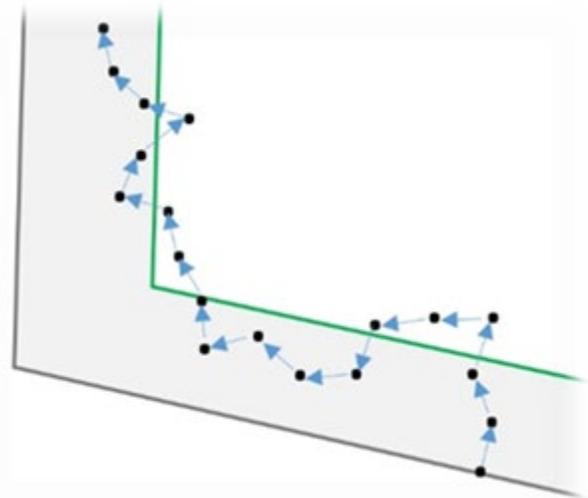
### INTRODUCTION

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.

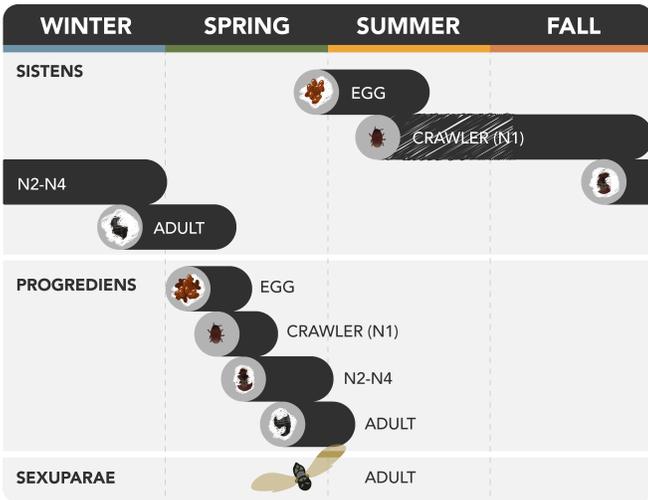
For both techniques, establish the sampling sites along a haphazard path through the stand, rather than along any one cardinal direction (for an example, see Figure 1). There should be at least a 25 metres spacing between each survey location, and two-thirds of all sampling locations taken within 50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



Created by the Invasive Species Centre in collaboration with the Canadian Forest Service.

**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 placed 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

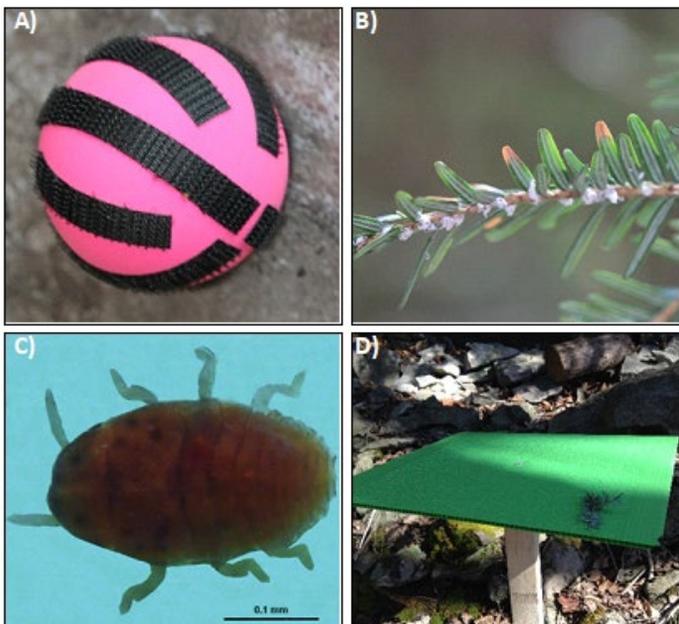
Using sticky traps to survey for HWA is simple and their construction and use is explained in a video (<https://www.youtube.com/watch?v=YfGaF7DbODo>). The sticky cards are made from rigid corrugated plastic cut to a 20 cm × 20 cm square. GLFC researchers developed this technique using green prism traps 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<sup>2</sup> 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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