

Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

*Hemlock
Woolly
Adelgid*

STANDARDIZING SAMPLING FOR DETECTION AND MONITORING OF HEMLOCK WOOLLY ADELGID IN EASTERN HEMLOCK FORESTS



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Standardized Sampling for Detection and Monitoring of Hemlock Woolly Adelgid in Eastern Hemlock Forests

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Introduction

The hemlock woolly adelgid (*Adelges tsugae*) is an introduced insect pest that has rapidly spread throughout many hemlock forests in the Eastern United States. Infestations are found from southern Maine to northern Georgia and west to eastern Tennessee (figure 1). The entire range of eastern hemlock (*Tsuga canadensis*) and the less common Carolina hemlock (*Tsuga caroliniana*) is currently at risk.

The natural spread of hemlock woolly adelgid is primarily by wind, birds, and small mammals; however, movement of infested nursery stock and other hemlock products has resulted in infestations occurring well outside the generally infested area (McClure 1990, Ouellette 2002, Burns et al. 2005). The rate at which hemlock woolly adelgid has spread is estimated to be 20-30 km (12-18 miles) per year

(McClure et al. 2001), and generally the rate of spread has been faster in the southern part of its range than to the north.

How quickly hemlocks die once infested is variable but generally ranges from 4 to 10 years or more (McClure 1991, Orwig 2002). In the northern part of its range, extremely cold winter temperatures will often reduce the number of surviving adelgids, particularly if temperatures fluctuate rapidly during susceptible life stages of the adelgid. Conversely, the milder winter temperatures in the southern part of its range result in higher population densities from year to year, which then hastens tree decline. Other stressors that affect how quickly hemlocks succumb include drought, site conditions, pre-existing tree health conditions, and abundance of other insect and disease pests.

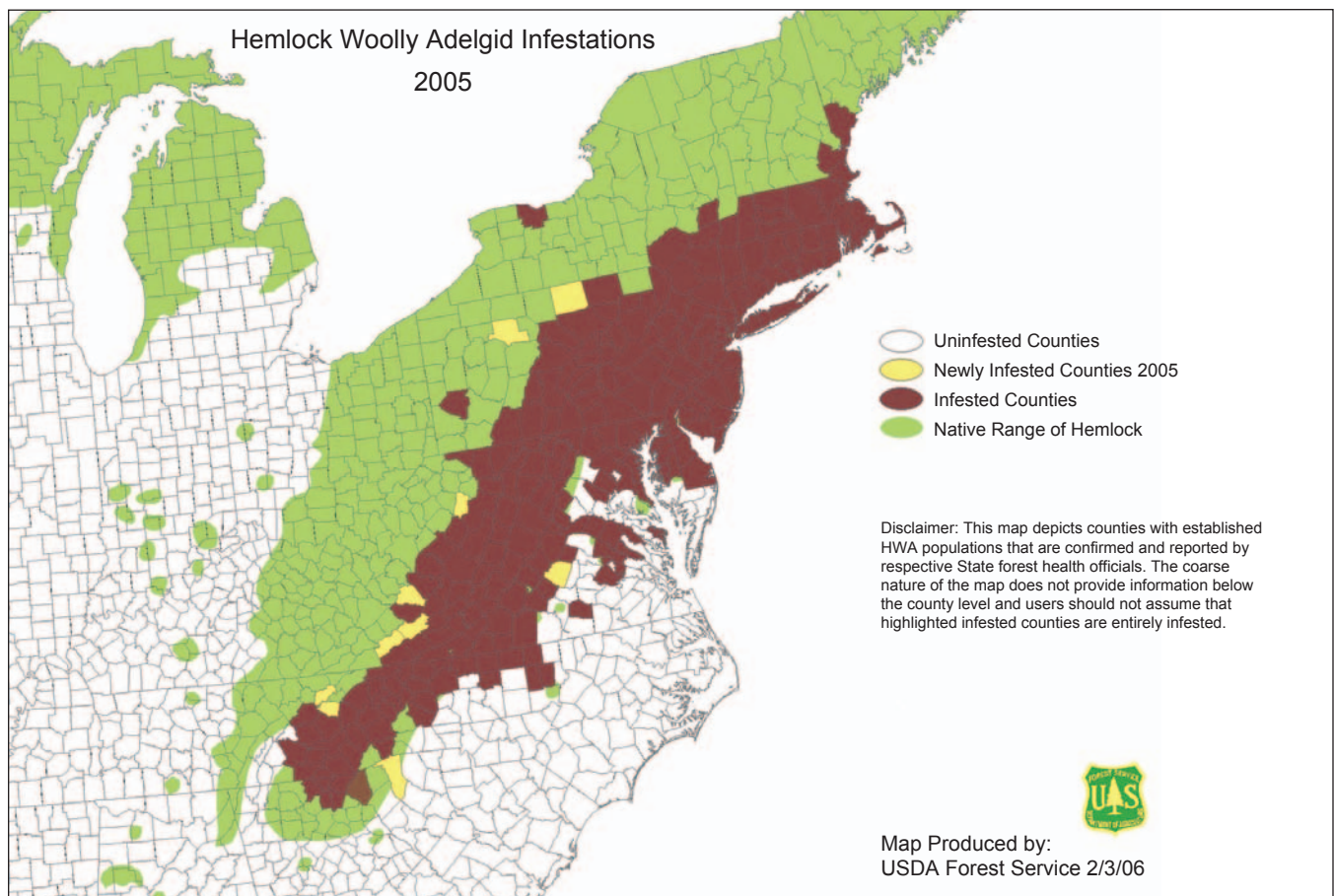


Figure 1. This map depicts the range of hemlock woolly adelgid in the Eastern United States as of 2005.

The purpose of this handbook is to provide resource managers with an efficient and effective sampling plan to detect, monitor, and assess the severity of a hemlock woolly adelgid infestation. By focusing on the percentage of infested trees within a stand, users can more precisely describe and communicate the severity of an infestation. Reliable infestation information allows greater confidence when making decisions about allocating resources for the management, restoration, and recovery of hemlock forests. This publication provides information to successfully sample hemlock trees for hemlock woolly adelgid and describes a sampling plan that uses a standardized method to quantify and communicate the severity of adelgid infestations.

Hemlock Woolly Adelgid Sampling Plan

The sampling plan measures the infestation level of the stand rather than of individual trees and does not involve laborious counting of adelgids. A strong relationship has been established between the percentage of trees infested and the number of adelgids found on branches (Costa 2005). The sampling plan gives users a quick and precise way to both detect and characterize the severity of an infestation by determining the percentage of infested trees. It is statistically based to provide specified degrees of reliability and precision and yet be flexible enough to accommodate various sampling goals (see Development of the Sampling Plan in the appendix). It was designed with input from resource managers so that it would be practical and straightforward to use.

By rapidly examining branches on 8 to 100 trees, infestations can be characterized, and as few as 2 percent infested trees can be detected with 75 percent reliability. The cutoff thresholds to stop sampling are based on optimum sample sizes to obtain a relative precision level of 0.25, the level commonly used for management purposes (Karandinos 1976). The sampling plan provides a minimum detection threshold whereby adelgids can be detected with 75 percent reliability in hemlock stands when less than 2 percent of trees are infested. Other levels of detection and reliability can also be selected if desired (table 1).

Detection versus Characterization of Infestations

Detection – The minimum detection threshold is the minimum percentage of infested trees in a stand required for the infestation to be found with a prescribed reliability. For instance, if no adelgids (woolly masses) are found after sampling 100 trees, then one can state with 75 percent reliability that the population of infested trees is less than 2 percent. Finding no woolly masses does not mean there are no adelgids in the hemlock stand, only that the population of infested trees is below the minimum detection threshold. If only 25 trees are sampled, approximately 5 percent of the trees would need to be infested to have a similar reliability (table 1). Selecting a minimum detection threshold involves balancing the risk of missing the infestation with the effort directed toward sampling trees; 100 trees may be a reasonable compromise for conducting surveys in large stands for detection purposes. If the goal is simply to detect the presence of hemlock woolly adelgid, then sampling can stop once the first adelgid is found.

Table 1. Maximum number of trees that must be examined to detect an infested tree by minimum detection threshold (minimum percent of infested trees) and reliability level (probability of finding a single infested tree). The shaded area encompasses the recommended 100-tree sample.

Minimum % Infested Trees in Stand	Reliability Level (%)			
	50	75	95	99
0.5	138	277	598	919
1	69	138	298	458
2	34	69	148	228
3	23	46	98	151
5	14	27	58	90
10	7	13	28	44
20	3	6	13	21

Characterization – Once hemlock woolly adelgid is detected, more information is often desirable, and the specified precision level (0.25) is recommended to determine the percentage of infested trees. The maximum number of trees to sample is set at 100, but as higher numbers of infested trees are encountered, substantially less sampling is required (see sampling datasheet in the appendix). When nearly all of the trees contain adelgids, only eight trees will need to be surveyed. However, because adelgid infestations tend to be clumped, it may be useful to begin anew in one of the other blocks and obtain a broader survey of the stand. The precision of the estimate is reliable down to 14 percent of trees infested; greater precision at low adelgid population densities will require a dramatic increase in the number of trees sampled with very little added information gained.

Developing the Proper Search Image and Timing of the Survey

Having a clear search image of what to look for is important for the success of any survey and paramount when conducting detection surveys or assessing lightly infested stands. Although adelgid size may vary, they are covered with a white woolly mass that is readily spotted along the underside of the terminal and lateral shoots of infested hemlock branches (figure 2).



Figure 2. White woolly masses of hemlock woolly adelgids are located at the base of needles along the terminal and lateral shoots of a hemlock branch. Careful examination of the background in this picture reveals extraordinary numbers of adelgids typical of advanced infestations. (photo credit – James Johnson, Georgia Forestry Commission, www.forestryimages.org).

The woolly masses are conspicuous because the whiteness of the wool contrasts with the darker branch and foliage (figure 3). Field personnel should develop a search image for individual or small clusters of woolly masses instead of the massive infestations seen in typical photographs found in many publications.



Figure 3. White woolly masses are extremely obvious in contrast to hemlock foliage and twigs. Their visibility increases in lower light conditions, such as during overcast days or in areas shaded by foliage. (photo credit – Chris Evans, The University of Georgia, www.forestryimages.org).

During mid-summer and early fall, adelgids aestivate on new hemlock growth as naked immature sistens; this adelgid stage is very small, has only a light ring of wool, and is more difficult to see with the unaided eye (figure 4). Although remnants of the previous generation of woolly masses can often be found, their appearance is less obvious and they can easily be missed. The optimum survey season is from late October to mid-July when the adelgid is actively growing and fresh wool is present (figure 5).

The white woolly masses surrounding each insect are almost invariably located at the base of hemlock needles. To the untrained observer, other things such as spittle bugs, caterpillar and spider webbing, scale insects, and even sap dripping from pine trees might be mistaken for the adelgid. However, these can be readily distinguished because of their general appearance, physical location, or both (figure 6). Any specimens with questionable features should be bagged and later examined microscopically, especially when surveying for infestations in new locales.



Figure 4. During summer, hemlock woolly adelgids aestivate in their first instar on the hemlock growth of the current year. These insects are very small and have only a small halo of wool, making them difficult to detect.

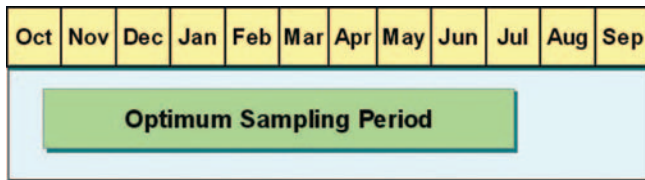


Figure 5. Sampling for hemlock woolly adelgid is best conducted when obvious woolly masses are present. Sampling during the summer months is possible but much more time consuming. Only remnants of the previous generation's woolly masses may be present, and detecting aestivating sistens would require the use of a hand lens. The times specified are a general guideline that may vary somewhat in different regions.

Examining the Tree

Trees are selected simply on the basis of having branches within reach without using a ladder or pole pruner. The underside of the first branch reached by the sampler is examined for any evidence of white woolly masses on the outer 1 meter of the branch. The sampler should select only live branches that are not completely devoid of needles and visually scan along the smaller twigs for woolly masses. The sampling plan only requires inspection for the presence or absence of woolly masses on either of two branches per tree. If no woolly masses are found on the first branch, a second branch on the opposite side of the tree is examined.

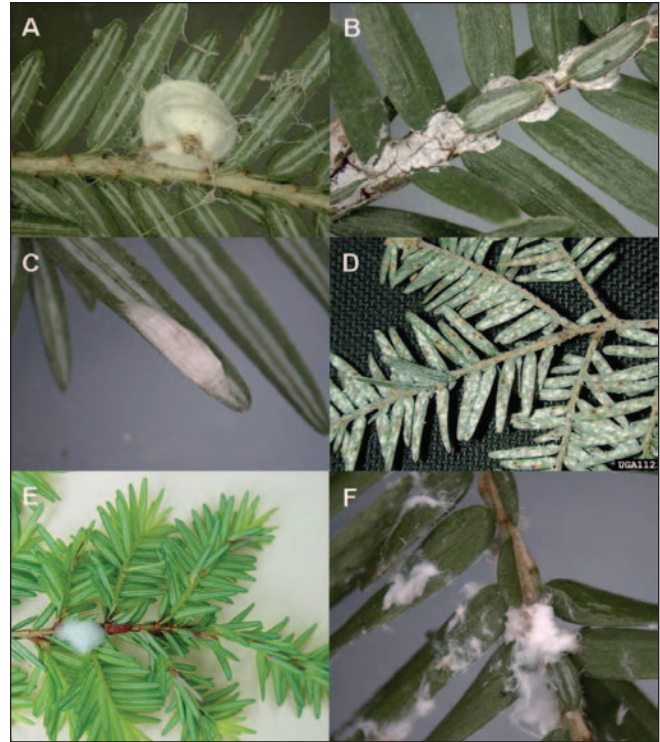


Figure 6. Other objects on hemlock foliage might be confused with hemlock woolly adelgid. A: Spider ovisac. B: Pine sap from an overhanging white pine. C: Oak skeletonizer on hemlock needle. D: Elongate hemlock scale *Fiorinia externa*. E: Froth from a spittle bug. F: Wool from white pine aphids blown from a neighboring tree. (photo credits – D. Ouellette, Maine Forest Service, for A, B, C, and F; Eric R. Day, Virginia Polytechnic Institute and State University, www.forestryimages.org for D; and S. Costa, University of Vermont, for E).

Counting individual adelgids is not necessary and impractical under field conditions because of their small size and the presence of often numerous adelgids at different life stages that are closely packed onto a hemlock twig. The sampling plan is designed such that a tree is considered infested even if only one adelgid is observed.

Sampling the Hemlock Stand

Only limited stand-level information, chiefly the presence of hemlock trees, is required in selecting sites to sample. However, the ultimate value of the result may be improved if information is available for developing larger-area sampling strategies and interpreting findings, e.g., aerial photographs, geo-referencing, and site attributes. Hemlock stands of 4 hectares (10 acres) or more with a somewhat strong

hemlock component are desirable because they provide sufficient trees to sample. In much smaller forest stands or stands containing only scattered hemlocks, the majority of trees would need to be examined. In this case, a more rigorous assessment of individual trees would be appropriate and more practicable.

The underlying research that went into developing this plan demonstrated that hemlock woolly adelgids have a clumped distribution (Costa 2005), as do many insects. This means that infested trees tend to be relatively close together in patches that extend across the landscape. The clumping diminishes as more trees in a stand become infested. For the sampler, this clumping tendency requires that the survey broadly cover the landscape to increase the chance of encountering an infested patch of hemlock trees. This is particularly important when attempting to detect low-level infestations. Quite often early infestations are first observed near roadsides or other travel corridors on individual or small clumps of trees, yet difficult to find in the interior of the stand. Therefore, an examination beyond a small number of trees grouped at the forest edge is particularly important if a reliable determination of the overall infestation level is needed for management decisionmaking.

After a brief reconnaissance, the stand is roughly divided into four blocks, but no measurements of stand or block sizes are required. The sampler simply identifies general areas or directions where sampling will take place. If establishment of global positioning system (GPS) coordinates within each block is desirable for mapping or future return to the stand, the first hemlock tree to be sampled in each block can be selected and the coordinates recorded.

Once the infestation status of the first tree is determined, a list of semi-random cardinal directions is consulted (see sampling datasheet in the appendix) and the sampler takes 25 paces (2 strides per pace) in the appropriate direction. Upon arrival at the next sample point, the nearest suitable tree is selected and examined. Sampling can stop once the first infested tree is found if the objective of the survey is solely for the purpose of detection. To determine the relative infestation level of the stand, sampling continues until either the appropriate stop level is reached or 25 trees

per block are sampled. Positive trees are indicated on the sampling sheet by maintaining a cumulative total of the infested trees observed.

The list of semi-random directions specified on the sampling sheet is designed as a guide to move the sampler throughout the hemlock stand (figure 7). However, there is flexibility in selecting how to cover a stand, and exactness in following specific cardinal directions is unnecessary. The objective is to ensure that the trees are well distributed within each sample block. When an obstacle occurs in the landscape, e.g., marshes, cliffs, or the hemlock stand ends, the sampler simply heads in an alternate direction. In cases where the hemlocks follow a stream, one can select a path that zigzags within the confines of the trees present. Again, the objective here is to get a representative sample, and in many cases the size, shape, and lay of a stand needs to be the guide. However, when using the prescribed approach that includes cardinal directions and fixed intervals in larger, more contiguous stands, the sampler ensures that the trees examined are sufficiently dispersed and will provide the representation necessary to adequately detect or characterize the infestation in a time-efficient manner.

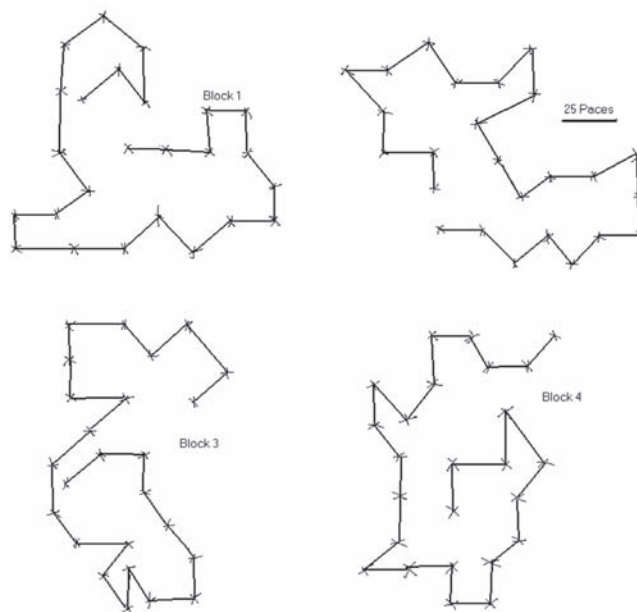


Figure 7. The semi-random paths provided on the sampling sheet would produce the paths outlined above if closely followed. The prescribed cardinal directions force the sampler to move about the stand, but other paths may be appropriate in irregularly shaped stands.

How Many Trees Are Examined?

- For simple detection, a single tree that is positive for hemlock woolly adelgid would suffice to establish its presence in an area. However, until one is found, as many as 100 trees are needed when using the recommended detection threshold of less than 2 percent at a 75 percent level of reliability (table 1).
- For estimating the percentage of trees that are infested within a stand, the plan calls for sampling 8 to 100 trees depending on how many infested trees are being found. The stop thresholds for terminating sampling are on the sampling datasheet.

Quick Start Instructions

This sampling method is best suited for stands 10 acres or larger, but smaller stands can be examined.

1. Briefly recon the site and roughly ascertain the location of four blocks that encompass the stand. Delineate block borders by selecting characteristics of the landscape using obvious features such as a stream or road where possible or general directions from the midpoint of the stand. Blocks should be large enough to limit significant overlap and ideally be at least 1.6 hectares (~4 acres) in size.
2. Go to a central point of the first block and arbitrarily select the first tree to examine. Trees should have two branches that can be reached from the ground, but a second branch on an adjacent or nearby tree is acceptable.
3. Select a branch and closely examine the underside of the terminal meter of foliage for the presence or absence of white woolly masses at the base of hemlock needles. If one or more adelgids are present, mark the datasheet with a 1 to indicate the presence of adelgid on that tree. If the goal is solely adelgid detection, then stop sampling. If the goal is characterization and quantification of the infestation, then go to step 5; if none were found, go to step 4.
4. If no woolly masses were observed on the first branch, select a second branch on the opposite side of the tree and examine as before. After the inspection is finished, mark the datasheet appropriately and go to step 5. NOTE: The data recorded is a running tally (sum) of the number of trees positive for presence of adelgid, i.e., 1 is added to the tally each time any adelgids are found on a tree.
5. Look at the datasheet to determine the direction to the next plot tree. Pace out approximately 25 paces (2 steps per pace) in the direction indicated and select the closest tree with branches that you can reach. Accuracy of direction and distance is not required; simply shoot the direction and go. NOTE: For stands that are narrow, smaller, or have a low density of hemlock, you may want to sample along a line trajectory, a “W” path, or even sample any available tree. The idea is not to be shackled to a fixed path, but to cover ground and get a representative sample.
6. Examine the tree for hemlock woolly adelgid as in steps 3 and 4.
7. If adelgids are detected, mark the datasheet by increasing the running tally by 1. If none were detected, re-enter the past tally number.
8. Repeat steps 5 through 7 until at least eight trees are examined and a decision can be made based on the criteria below:
 - a. If NO woolly masses were found, repeat steps 5 through 7 until adelgids are detected or 100 trees are sampled. Go to *b* once any adelgids are detected or stop sampling if no adelgids are found after 100 trees are sampled.
 - b. If the tally count is less than the stop threshold, then repeat steps 5 through 7 until the threshold is reached, entering new blocks as required after each 25 trees.
 - c. If the tally count is less than the stop threshold after 100 trees, then stop sampling.
 - d. If the tally count is greater than the stop threshold, then stop surveying the stand.
9. Compute the level of infestation as indicated below:

Percent infested trees = (number of infested trees) / (number of trees inspected) × 100

Frequently Asked Questions

“What do I do if the site is long and narrow; do I have to stick with the cardinal direction scheme?”

No, you can go off on a tangent line or use some other scheme that allows you to get a representative sample, while still spacing out your samples.

“What do I do if the plan says go northeast and there is a lake?”

Head in a new direction when an obstruction is encountered.

“What do I do if there was no tree at the end of 25 paces?”

Pick the closest suitable tree.

“What do I do if foliage quality is low and the needles are sparse?”

Sample anyway, unless no needles are present; the poor quality might be due to an infestation.

“What do I do if the hemlock stand ended?”

Head back into the stand.

“What do I do if there are no branches on the opposite side of the tree?”

Pick the branch farthest from the one already sampled.

“What do I do if the blocks are overlapping and the forest is being re-covered?”

Walk back into the current block and resume sampling.

“Why avoid sampling in late summer?”

The adelgids are small, have no woolly coat, and are difficult to see.

“What is the consequence of stopping sampling before reaching the appropriate threshold in the plan?”

The population estimate will be less precise.

“Why doesn’t it matter if the adelgids are dead or alive?”

The presence of white woolly masses indicates that hemlock woolly adelgids have been and will likely continue to be in the area.

Appendix

Development of the Sampling Plan

The hemlock woolly adelgid (HWA) sampling plan was founded on an extensive data set (1,700 trees across 17 hemlock stands) collected by Joe Brown, an intern in the Harvard Forest Summer Research Program in Ecology, Petersham, MA (Brown 2003). A standard statistical approach was taken in which progressively larger areas of the lower branches of hemlock trees were sampled, i.e., five branchlets on each of two branches, a 1-meter terminal section of two lower branches on opposite sides of the tree, and all the lower branches of a tree. Additionally, counts of white woolly masses were taken from each of the branchlets examined; the count data were collected so that the relative size of adelgid populations within a stand could be correlated with the proportion of trees that were infested. Four blocks of 25 trees were examined within each stand. Trees within a stand were randomly selected for sampling by taking 25 paces in random cardinal directions and selecting the closest available hemlock tree.

The three sampling methods provided similar results, and infestation levels ranged from 0 to nearly 100 percent trees infested within the 17 stands examined (figure 8). The two-branch sample was selected to create a more uniform sample among trees of different sizes and to give the sampler a finer focus in searching for adelgid white woolly masses, especially when densities are low. Calculations of minimum detection thresholds and optimum sample sizes were conducted based on the two branch per tree sampling method.

Minimum detection thresholds are purely probabilistic and were originally developed to characterize the chances of finding defective products in manufacturing quality control (Venette et al. 2002). The basic relationship is between the number of items that need to be examined and detection of the subject of interest when a specified percentage is present, whether it be adelgid-infested trees or defective light bulbs. The results from this analysis are in table 1, which portrays the number of trees required to detect an infested tree at various probabilities and infestation levels.

Stop thresholds that allowed a sampling precision level of 0.25 were established by first calculating optimum sample sizes for characterizing the presence or absence of adelgids, i.e., binomial sampling (Karandinos 1976). From this, stop thresholds for samples of 8 to 100 trees were determined based on the infestation that could be detected at the intended relative precision of 0.25. These stop thresholds are listed on the sampling datasheet.

Analysis of the variance to mean regression, viz Taylor's power law (Taylor 1961), found a significant ($P \leq 0.05$) relationship between these parameters based on the summed count data averaged by tree (Taylor's $a=7.96$ and $b=1.499$ with $r^2=0.96$). When the Taylor values were incorporated into the Wilson and Room (1983) model for predicting the percentage of trees infested, a significant relationship ($P \leq 0.05$; $r^2=0.91$) was found between the predicted percent trees infested based on the count data and observed percent trees infested found in each site using branch samples. This suggests a predictable relationship between count data and percent trees infested that will prove useful in research related to HWA population biology. Contact S. Costa for further information.

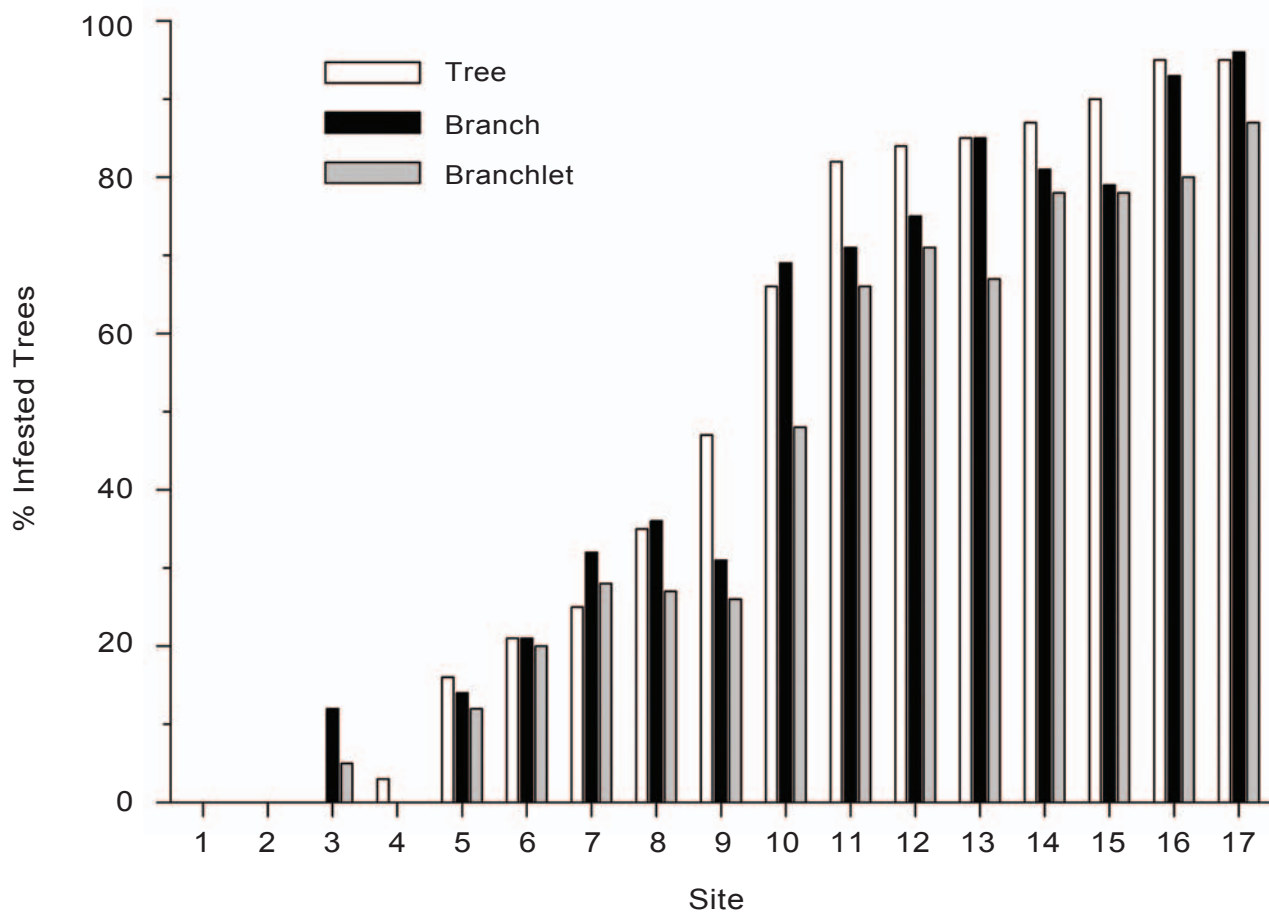


Figure 8. This graph illustrates the percentage of trees infested using three sampling methods in each of the 17 stands used to develop the sampling plan. Increasingly larger sample sizes of the lower canopy were used.

Hemlock Woolly Adelgid Sampling Datasheet

Date:		Location:			Sampler:			Comments: on reverse side			
Path Direction	Tree	Sum HWA Trees	STOP \geq Threshold	Direction	Tree	Sum HWA Trees	STOP \geq Threshold	Direction	Tree	Sum HWA Trees	STOP \geq Threshold
Block 1	1		n/a	SW	35		11		69		13
NE	2		n/a	W	36		11		70		13
SE	3		n/a	SW	37		11		71		13
N	4		n/a	NW	38		11		72		13
NW	5		n/a	NW	39		11		73		13
SW	6		n/a	NE	40		11		74		13
S	7		n/a	N	41		12		75		13
S	8		8	SW	42		12		Block 4	76	13
SE	9		8	W	43		12		N	77	13
SW	10		8	NW	44		12		E	78	13
W	11		8	SW	45		12		N	79	13
S	12		8	W	46		12		SE	80	13
E	13		8	SE	47		12		SW	81	13
E	14		8	S	48		12		S	82	13
NE	15		8	E	49		12		SW	83	13
SE	16		8	S	50		12		S	84	13
NE	17		8	Block 3	51		12		W	85	13
E	18		8	NE	52		12		N	86	13
N	19		9	E	53		12		W	87	13
NW	20		9	S	54		12		W	88	13
N	21		9	SE	55		12		NE	89	13
W	22		9	SE	56		12		N	90	13
S	23		9	S	57		13		N	91	14
W	24		10	W	58		13		NW	92	14
W	25		10	NW	59		13		N	93	14
Block 2	26		10	S	60		13		SE	94	14
E	27		10	NW	61		13		NE	95	14
SE	28		10	NE	62		13		N	96	14
NE	29		10	W	63		13		E	97	14
SE	30		10	NW	64		13		SE	98	14
NE	31		11	N	65		13		E	99	14
E	32		11	NE	66		13		NE	100	14
N	33		11	NE	67		13		STOP		
N	34		11	W	68		13				

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