Binomial Sampling Plan for *Adelges tsugae* on Eastern Hemlock

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ABSTRACT

The hemlock woolly adelgid is an exotic insect pest of eastern hemlock, *Tsuga canadensis* (L.) Carr., and Carolina hemlock, *T. caroliniana* Engelm., in the eastern United States. Despite a significant amount of research and management of *Adelges tsugae* (Homoptera: Adelgindae), no statistically based sampling method exists for this tiny and numerous pest. Binomial sequential sampling is currently very popular because it is one sampling plan that gives significant savings of cost and time. We developed an adelgid binomial sequential sampling (HWA BSSP) plan for *individual hemlock trees* at action thresholds of 10% and 30% of new shoots infested with at least one sistens. The HWA BSSP was validated with 210 datasets (trees) sampled across the eastern United States. Compared to a whole sample method (avg_n = 228 shoots per tree) that would be used in lieu of the HWA BSSP, the average number of shoot samples inspected to give acceptable levels of accuracy was 81% and 86% lower at the 10% and 30% thresholds, respectively. The plan requires from 12 to 15 minutes to complete per tree.

We also developed a method of assessing *A. tsugae* infestations *in situ* with the original HWA BSSP. This method involves randomly selecting four lower crown branches per tree and counting the number of shoots infested on the first five new shoots on each branch. This count is compared to the HWA BSSP and infestation levels are classified accordingly if the count is lower or greater than the lower and upper stop sampling boundaries, respectively. If the count remains within the boundary, the number of new shoots infested by at least one adelgid must be counted on the next five new shoots on the same branches, etc. After each round of counts, the total value is compared to the appropriate threshold plan and the infestation is classified (low, high, or continue sampling) accordingly. If 80 new shoots have been examined (20 per branch) without reaching a classification decision, the infestation is classified as indeterminate and should be re-evaluated in six months. We have sampled 48 trees with this method, and infestations were classified with 100% accuracy. This method required an average of two minutes to classify infestations on individual trees, provided lower crown branches were accessible and had sufficient new growth.

KEYWORDS

Binomial, sequential sampling, hemlock, *Adelges tsugae*.

Sampling for Detection and Monitoring of Hemlock Woolly Adelgid within Hemlock Stands

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ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae*) has been spreading through eastern United States for over 50 years, destroying hemlock forests and dramatically changing the landscape. However, the lack of an efficient sampling plan for detecting new infestations and quantifying the percentage of infested trees hampers foresters and researchers. A survey of 1,700 trees in 17 sites with a wide range of infestations forms the basis of the hemlock woolly adelgid (HWA) sampling plan. This is a statistically based plan that allows for defined reliability of population estimates. Yet the plan is relatively straightforward in its execution and is flexible enough to accommodate various sampling goals: the forest practitioner surveys two ground-level branches per hemlock tree for the presence or absence of white woolly masses of adelgid on 1-meter terminal sections; between eight and 100 trees are sampled depending on how many positive trees are found. This allows determination of the percentage of trees infested at the specified precision level (0.25). No sampling plan can ever confirm that a hemlock stand is free of HWA. However, after sampling 100 trees and finding no HWA, the conclusion made with 75% reliability using this plan is that HWA infest less than 2% of the trees. Various levels of assuredness of detection are outlined relative to sampling effort.

KEYWORDS

Binomial, sampling, detection, monitoring, range.

INTRODUCTION

For over 50 years, the hemlock woolly adelgid (*Adelges tsugae*) has been spreading through eastern United States (Cheah *et al.* 2004), and yet no statistically based sampling plan has been developed that allows for the efficient detection and characterization of infestations. A standardized plan would facilitate monitoring of range expansion, aid in directing and gauging the success of management efforts, and serve as a valuable research tool. The primary goals of the hemlock woolly adelgid sampling plan (HSP) are to determine if HWA are in a forested stand (detection) and, if present, the level of infestation (characterization). Many sampling

plans are created and never widely adopted. For a plan to be adopted, foresters expect the plan to be simple, practical, and have a defined reliability.

Routine sampling of branches above the lower crown (outside of arms reach) is inefficient when relatively large numbers of trees must be examined. For this reason, the HSP is based on sampling lower branches. However, the question might be asked whether or not these lower branches are representative of HWA presence throughout the canopy. Fidgen (personal communication) examined both lower and mid-crown branches of 78 hemlock trees for the presence or absence of HWA. He found that, in over 60% of the trees, both crown strata were infested. When only one stratum was infested, it was invariably the lower crown, which tended to occur with lower density populations. Therefore, sampling lower branches, when available, should give an indication of a trees infestation status. Although counting adelgid on hemlock trees is not impossible under field conditions, routine counting for sampling is not practical because of their small size and the presence of multiple life stages.

PLAN DEVELOPMENT

A binomial sampling plan where the presence or absence of HWA white woolly masses is assessed forms the basis of the general sampling plan structure. Development of the HSP was facilitated through funding from Harvard Forest Summer Research Program in Ecology. The intern, Joseph Brown of Holyoke Community College, collected an extensive dataset that formed the foundation for creating the HSP. 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. This data was collected so that the proportion of infested trees could be related to the relative size of HWA populations within a stand. Four blocks of 25 trees were examined within each of 17 sites for a total of 1,700 trees surveyed. Trees within a stand were randomly selected for sampling by taking 25 paces in random cardinal directions and selecting the closest available hemlock tree.

A thorough treatment of the foundation data is not the subject of this paper. However, mention of certain results is necessary for clarity and to instill user confidence in the framework from which the HSP was developed. Infestation levels within the 17 stands examined ranged from 0 to nearly 100% trees infested, with the three sampling methods providing similar results (Figure 1). While sampling of all lower branches tended to indicate higher infestation levels, 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.

A variety of equations were either applied to the data or used to define sampling parameters. Taylor's power law (Taylor 1961) analysis of the variance to mean regression of the summed count data averaged by tree found a significant ($P \leq 0.05$) relationship of these parameters (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 percentage trees infested, a

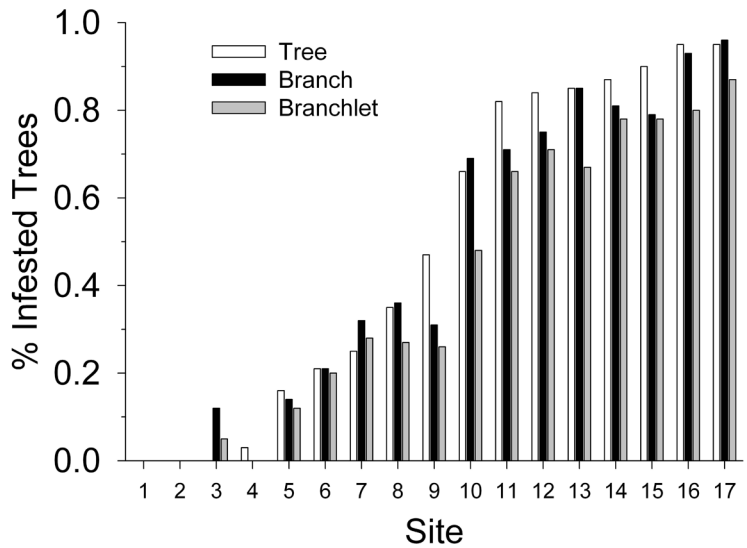

Figure 1. The percentage trees infested in each of the 17 sites used for development of the hemlock woolly adelgid sampling plan when sampled using either branchlets, the terminal meter of two branches, or all lower canopy branches.

significant relationship ($P \leq 0.05$; $r^2 = 0.91$) was found between the predicted percentage of trees infested based on the count data and observed percentage of trees infested found in each site using branch samples. This suggests a predictable relationship between count data and percentage of trees infested.

More relevant to the goals of sampling for detection and characterization of adelgid populations are calculations of minimum detection thresholds and optimum sample size. Minimum detection thresholds are purely probabilistic and don't require use of the field data (Venette et al. 2002). Originally developed for quality control, they are applied to define the probability of detecting the subject of interest at a given level of frequency and specified number of samples, whether it be infested trees, bad light bulbs on an assembly line, or pests in shipments of imported bananas. Table 1 portrays the number of trees required to detect an HWA infested tree at various probabilities and infestation levels. Optimum samples sizes were calculated for binomial sampling at a precision level of 0.25, which is standard for purposes of assessment (Karandinos 1976). The values for optimum sample size were then used to calculate sampling stop points based on the number of infested trees being found and a relative precision of 0.25. The stop points are inserted on the sampling data sheet (available upon request).

HEMLOCK WOOLLY ADELGID SAMPLING PLAN (HSP)

The HSP allows for the standardized detection and characterization of HWA infestations at prescribed levels of reliability and precision. By sampling up to 100 trees, HWA infestations within a stand can be detected with 75% reliability when nearly 2% of trees are infested. Efficient determination of the percentage of trees infested can be accomplished by sampling from eight to 100 trees depending on the number of infested trees being found. A complete, step-by-step description of the sampling plan, including a data collection sheet, is available upon request. The outline provided below provides an overview of the plan's principal components.

Table 1. The maximum number of samples required at different percentage probabilities of finding a single infested tree to provide the minimum detection thresholds indicated (minimum percentage of infested trees).

Minimum % Infested Trees	Maximum # Trees to Detect an Infested Tree at Probability (%)			
	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

STAND SELECTION

Only limited stand information is required in selecting sites to sample. Although aerial photos would be useful for initial identification and delineation of stands, they are not essential. Generally, more available information (geo-referencing and site characteristics) enhances the ultimate value of the result. Stands four hectare or more in area with a somewhat strong hemlock component are desirable because they provide sufficient trees to sample. Much smaller sites or those with sparse populations of hemlock can be examined, but the selection of trees and thresholds for stopping sampling may not be as appropriate. Obviously there is a point when you would no longer benefit from the plan (i.e., when there are only a few trees or the majority of trees would have to be examined). In this case, a more rigorous assessment of individual trees may be practicable.

WHAT TO SAMPLE

After a brief reconnaissance, the stand is roughly divided into four blocks, but no measurements of stand or block sizes are required. The first hemlock tree to be sampled is then selected. No consideration of tree health is made in the selection except that sample branches are not completely devoid of needles. Trees are selected simply on the basis that they have branches within reach. The underside of the terminal 1 meter of the first branch encountered is examined for any evidence of white woolly masses indicative of HWA. Survival status of the HWA is unimportant and no counting is required. If no HWA were found on the first branch, a second branch on the opposite side of the tree is selected for examination. Evidence of HWA may include large ovisacs, immature HWA, or even scars of woolly masses. The white woolly masses are located at the base of needles and are readily distinguished from other organisms on hemlock, such as spittle bugs, caterpillar and spider webbing, scale insects, etc. Findings of questionable identification should be bagged for later microscopic examination, especially when infestations in new locales are found. The number of HWA on a

tree is lower when fewer trees are infested. A few trees may have many HWA (as in the typical photos), but most trees will have few or no HWA, particularly when few trees are infested. Visually scanning along smaller twigs is an useful tactic for espying HWA.

Once the infestation status of the first tree is determined, a list of semi-random cardinal directions is consulted, the sampler takes 25 paces in the appropriate direction and then selects the nearest suitable tree. There is flexibility in selecting trees to cover a stand. The primary objective is to get a representative sample and, in many cases, the size, shape, and lay of a stand needs to be the guide. However, using a prescribed approach such as cardinal directions and fixed intervals ensures sufficient trees are examined in a time-efficient manner. After 25 trees are sampled in a block, the surveyor moves to the next block and samples additional trees.

HOW MANY SAMPLES

For simple detection of HWA within a stand, a single tree that is positive for HWA would suffice to establish its presence in an area. However, if no HWA are found, as many as 100 trees must be examined, depending on the specified infestation threshold and desired assurance of detection (Table 1). Often, data on the level of infestation within a stand are wanted. For estimation of the percentage of trees infested, the HSP calls for sampling from eight to 100 trees for the presence or absence of HWA depending on how many positive trees are found. As the sampler moves from tree to tree, a running tally is made of positive trees found, and this sum is compared with a threshold on the sampling datasheet. Once the threshold is achieved sampling stops. The percentage of trees infested is simply calculated by dividing the number of positive trees by the number examined trees and then multiplying by 100.

WHEN TO SAMPLE

This plan is intended for use when HWA with a white woolly coat can be observed. The optimal sampling period is from early winter, when HWA begin putting on their woolly coat, until early summer, when HWA sistens settle on new growth. During summer the sisten generation is in the first instar and lack a woolly coat, and are therefore difficult to see. They aestivate until mid fall when they resume growing and develop a woolly coat. The utility of HSP for sampling during the aestivation period is under investigation.

PLAN ADOPTION

The first workshop for training forestry personnel on use of the HSP was conducted during the spring of 2004 in Massachusetts. Representatives from Massachusetts, Maine, New Hampshire, New York, and Vermont attended and rated the usefulness of the plan as “high.” However, the success of any sampling plan is gauged by its adoption. Already, the HSP has been used on at least 97 sites in New York, and the U.S. Forest Service has examined 92 stands in Alleghany National Forest and 50 stands in Monongahela National Forest. The 92 Alleghany stands took three crew members two weeks to complete. A survey of properties associated with Harvard Forest, Petersham, Massachusetts, found relatively low populations in stands previously thought to be uninfested.

The availability of a standardized sampling plan offers many opportunities for its incorporation into activities associated with HWA detection, surveillance, and management. Although plan validation and characterization are ongoing, the general utility of the HSP is already evident, state and federal agencies are collecting geo-referenced data and using handheld computers for data management. At Harvard Forest and elsewhere, its utility as a research tool is becoming evident. Currently, no decision thresholds for initiation of management are tied to the plans results. The apparent flexibility and straightforwardness of application of the HSP should allow it to be widely employed.

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