FHP TECHNOLOGY DEVELOPMENT PROJECT PROPOSAL

PROJECT STATUS: New, not previously funded.

PROJECT NUMBER: R8-2000-exotic_New_Proposal

PROJECT TITLE: Improved Sampling Procedure for Population Estimation of the Hemlock Woolly Adelgid on Eastern Hemlock Trees

SUBJECT: Insect management, exotoc pests, hemlock woolly adelgid, monitoring pest, biological control, sampling procedures, hazard & risk rating

PROJECT OBJECTIVES:

1. To develop a binomial sampling procedure that estimates hemlock woolly adelgid (HWA) density on a per tree basis. Such a protocol would be simpler than the currently used procedure requiring total counts on *x* number of twig sections.
2. Evaluate the role of tree and site measures as potential covariates for integration into the binomial sampling procedure.
3. Develop a website that calculates the population estimates for the user.

BRIEF DESCRIPTION OF PROJECT: A previous effort to create a binomial sampling procedure for estimating HWA population density showed great promise. Its flaw was the inability to use the same underlying relationship, between proportion of twigs infested and number of HWA with a predetermined threshold, from one site to the next. We propose to build on this first effort and devise a procedure that can be used for all hemlock stands infested with HWA. We will do this by sampling more sites across the geographic range of HWA and collecting additional data associated with the health of infested trees. These tree or site measures may serve as covariates that can be used to stabilize the relationship from site to site and result in the creation of either a standard protocol for all sites, or a modifiable protocol that depends on certain site or tree characteristics. The data collected may allow us to develop a risk-rating method for trees and help initiate the process of hazard-rating hemlock stands. Data collected from plots will be used to build the statistical model from which the procedure will be based. The model will then be validated using data from randomly selected HWA infested stands. A website will be developed that will allow for data input and calculate population estimates.

FHP PERSON WHO WILL LEAD THE PROJECT: James R. Rhea, Asheville, NC.

PRINCIPAL INVESTIGATOR: Dr. Scott M. Salom, Associate Professor, Dept. of Entomology, Virginia Tech, Blacksburg, VA Tech, Blacksburg, VA.

COOPERATORS: Dr. Harold Burkhart and Ralph Amateis, Virginia Tech; Dr. John Karish and Richard Evans, USDA, NPS; Dr. Richard Reardon, USDA Forest Service, FHTET.

JUSTIFICATION: The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), is an introduced insect pest that is killing large numbers of eastern hemlock trees, *Tsuga canadensis* (L.) Carr throughout the eastern United States. As a result, this ecologically important tree species is seriously threatened. Currently no operational control effort is available for suppressing this pest. Yet, recent evaluations of imported natural enemies suggest that their release

may have a positive impact and reduce HWA densities on attacked trees (McClure and Cheah 1999). Because of HWA's microscopic size and frequently high densities, population estimates are extremely labor intensive and time consuming. Improved sampling procedures are needed to aid in monitoring population over time and to determine the impact of pest management tactics employed such as the release of natural enemies. Gray et al. (1998) report on a recent attempt to develop a binomial sampling procedure for estimating mean HWA density on individual trees. This procedure entails noting the presence of $> T$ HWA or $\leq T$ HWA on a specified number of twigs, where T is a predetermined number. Using a negative binomial model, a significant relationship between the proportion of twigs sampled containing T HWA and population density was observed at all sample sites in Virginia and Pennsylvania and for both sistens and progredien lifestages. The slope of this relationship was the same for all sample sites and lifestages. Only the point of origin on the y-axis differed. Thus, the overall relationship differed from site to site and between generations. Gray et al (1998) hypothesized that the changing health of the infested stand over time was the basis for the different relationships observed.

We propose to investigate this hypothesis and in the process hope to devise a procedure that stabilizes the relationship enough to allow for its use as an operational sampling tool. This can be done by expanding the data set on which the binomial procedure is based and by identifying key host tree or site measures that are linked to the changing stages of tree health over time.

URGENCY: Currently, the only treatment for HWA in forest settings is the release of biological control agents. One species, *Pseudoscymnus tsugae* is being released in limited fashion (ca. 15 release locations in 1999). At least three other species, two *Scymnus* spp. and *Laricobius nigrinus*, also show good prospect for release. Eventually the number of releases per year will increase as the new agents become available and their rearing becomes more efficient. Current approaches to evaluating these releases is very time consuming. It is essential that we be able to collect HWA population data quickly and accurately to determine the efficacy of each natural enemy alone as well as a potential complex of predators. If the procedure developed is accurate from site to site, it will likely replace the currently used procedure that requires total counts of HWA on x number of twig sections per tree.

The new sampling procedure may also improve our ability to choose suitable release sites for the natural enemies. Currently, researchers are determining the best infestation and stand conditions for releasing *Pseudoscymnus*. The sampling procedure may reduce the time necessary for choosing stands that meet the proper release criteria and provide a more objective explanation to landowners whose sites are not chosen for the releases.

Use of the sampling procedure will be incorporated by managers or scientists interested in quantifying the effect of other management treatments or control procedures developed for HWA.

LINK TO NATIONAL FHP TECHNOLOGY DEVELOPMENT PRIORITIES: This project fits squarely into two of the four national program priorities. Improved sampling for HWA will reduce the time needed for evaluating the success of natural enemy releases (priority 1). The data obtained from various tree and site measures used to develop the sampling procedure can become the basis for risk rating of hemlock trees or hazard rating of stands containing a significant hemlock component (priority 2).

SCOPE OF APPLICATION: The sampling procedure will be used by entomologists throughout the geographic range of HWA. It will replace the current method of complete counts of HWA on twigs for evaluating population increases into new areas, impacts of natural enemies at sites where they have been released, and impacts of other control tactics, such as pesticide applications. With the time saved, it will allow entomologists to sample a greater number of sites.

RESEARCH BASIS: The estimate of pest population size (or density) is an important component of most integrated pest management (IPM) and many research programs. Efforts to quantify the ecological impact of HWA requires reliable population estimates. Hemlock woolly adelgid management decisions should be made partly on the basis of population levels; and evaluation of management treatments rely on precise estimates of population density. However, obtaining acceptable estimates by counting HWA individuals is difficult because of their very small size and their frequently very high density. Current sampling procedures require an extremely arduous full count method for estimating mean HWA population density. It involves counting the number of HWA on four 25 cm branches. This requires bringing samples back to the lab and spending 8 h of microscopic evaluation for every 1 h of sampling in the field.

A less time-consuming procedure that may be applied to HWA is binomial sampling. It relies on the inherent dispersion of the pest on its host, and the concommitant relationship between the proportion of samples with more than a predetermined pest threshold and the mean pest density. If the predetermined threshold is zero, pest density is estimated from the presence or absence of pests in the samples (Southwood 1978). Binomial sampling should be highly suited to a pest such as HWA. Obtaining population density estimates by counting HWA individuals is difficult because of their size (<1 mm), their frequently high density (>9/cm of twig length), and their clumped dispersion within a tree.

Gray et al. (1998) recently attempted to develop a binomial sampling protocol based on data taken from both generations in each of two states (Pennsylvania and Virginia). However, significant differences in the density vs. proportion infested relationship were detected between the individual state - generation combinations when a simple presence vs. absence classification was used. They were able to reduce the differences between states and generations by increasing the pest threshold to 3 (ie. ≤ 3 vs. > 3), yet an overall general relationship remained elusive. The absence of a general relationship applicable in any location would obviate the utility of the binomial method. At this point additional effort is needed to develop an operational sampling method.

The status of individual trees in a stand, with resepct to severity of infestation, may affect the relationship between the proportion of twigs containing HWA and the overall density of HWA on the tree. McClure (1991) describes a density-dependent relationship between overall tree health and infestation level of HWA on a host tree. This leads to the hypothesis that individual tree or site measures may act as covariates that improves density estimation, with minimal additional inputs.

The methods described below are, in part, designed to test this hypothesis and provide a user-friendly procedure that will allow resource managers and scientists to sample for HWA and make reasonable population estimates.

METHODS: Objective 1. Plots for sampling HWA density will be established in geographically distinctive forest stands where HWA-infested hemlock is a significant species component. We will be searching for sites in which trees have < 33, ≥ 33 and ≤ 66, and $> 66\%$ new shoot growth. The categories represent severely impacted, moderately impacted, and lightly impacted trees, respectively.

New shoot growth is felt to be a better measure of infestation status than % defoliation which is a difficult and subjective measure to estimate.

Plots for sampling HWA density on trees will be established at 6 locations that span the insect's north-south geographic range (Fig. 1).

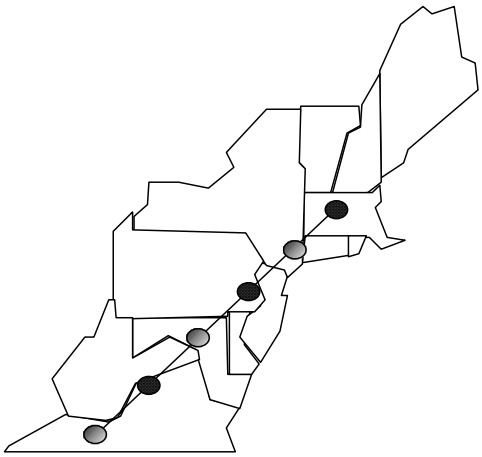

Fig. 1. Sample transect for collecting HWA population density data. Dark samples represent year one sample sites and light circles represent year 2 sample sites.

Because of the long time it takes to process the samples, the short length of time samples remain viable, and the need to synchronize sampling among locations, it is unreasonable to try to complete all sampling at one time for each generation. Therefore, sampling will take place over two years. Fall-winter and spring generations of HWA will be sampled in fall 2000 and spring 2001, respectively, at locations represented by darkly shaded circles (Fig. 1). Fall-winter and spring generations of HWA will be sampled in fall 2001 and spring 2002, respectively, at locations represented by lightly shaded circles. The plots and trees within plots used to sample fall-winter generations of HWA will be revisited to sample spring generations.

Four trees will be sampled within each site. Four branch tips will be cut from each of two heights. From each branch tip, eight twigs (branch tip to first node) will be randomly selected. Each twig will be examined with the naked eye for presence of HWA. The number of HWA will then be determined with the aid of a dissecting microscope.

Objective 2. To identify potential covariates, the following measurements will be made from each plot:

Tree Measures	**Forest Measures**
1. % dead branches	1. forest type
2. % of branches with new growth	2. basal area of hemlock
3. mean length of most recent shoot growth	3. basal area of other species
4. tree age	4. slope
5. tree height	5. aspect
6. dominance	
7. live crown ratio	
8. radial growth over the past 5 years	
9. radial growth over the past 10 years	

Crown condition measurements are based on protocol developed by the USDA Forest Service, Forest Health Monitoring (Millers et al. 1992). All these measures will be included in a semi-saturated (only some of the interactions) general linear model estimated by Proc GLM (SAS 1990) for each plot by generation combination. The contribution of each main effect and interaction will be evaluated by Type III sums of squares. Measures that add significantly to the estimate and and help stabilize the relationship from plot to plot will be incorporated into the procedure. None of the measures listed above are considered difficult to make, and therefore should not add much burden to

the sampling procedure.

Objective 3. A website will be created that 1. provides instructions on how to use the sampling procedure and 2. calculates the population density estimates from user inputs. The calculations will be generated using a cgi (common gateway interface) program that will interface with an HTML form. It will be possible to input level of precision desired, HWA generation, sample numbers, and covariate measures. The resulting output should be the popuation estimate. It may be more appropriate to develop an applet using Javascript® that can make the calculations without going back to the server. The advantage of this approach is that error checking can be built into the script. We will determine later which approach is best for our needs.

MEASURE OF SUCCESS: Our goal is to devise an easy to use sampling plan that can provide reasonable estimates of HWA populations from one site to the next. Based on Gray et al. (1998), it is likely that we will need to devise a separate equation for both HWA generations. Also from Gray et al. (1998), presence-absence in its simplest form will need to be modified to accept presence of *T* or more HWA on a sample twig, where *T* is a predetermined threshold (ie. 2, 3, 4, or....). It is also likely that covariate measures will have to be included as well. With these additional measures, the once simple presence-absence sampling procedure does become more complicated, yet still provides managers and researchers with an easier procedure than the one currently used.

The statistical model we generate for each HWA generation will be validated during the 3rd year of the project. We will collect data in a number of hemlock stands at different levels of infestation described in the Methods section. We will determine how many samples are needed to obtain an acceptable level of precision. If we succeed, we will make this tactic available to all that are interested.

Website critiques will be requested from users. I will use those comments to adjust and modify the site as needed. If we use a cgi program, we will be able to monitor the use of the population estimator more closely and see from the server if users are having success accessing and using the program.

PRODUCTS: A modified binomial sampling procedure will be made available to potential users. Instructions for its use will be made available in a Forest Service publication and from a website that will also calculate the estimates for the user.

PUBLICATION: The results from this work will be written up for peer-reviewed journals. The description of the procedure will be written as a Forest Service Handbook publication.

TECHNOLOGY TRANSFER: The Forest Service will publish and distribute the handbook. The website will be maintained on a Virginia Tech server that is available to all. Forest Health Entomologists will use the procedure and train their clientele on how to use it.

PROJECT DURATION: 3 years (2000 - 2003).

LONG TERM BUDGET: The STDP will provide 75% stipend support for a graduate student, some wages for technical support, travel expenses, materials & supplies. The National Park Service will provide 25% of the stipend funds for two years since they will allow us to pay tuition. FHTET will provide additional funds for technical staff support for two years; Virginia Tech will handle the indirect costs @ 45% of the STDP.

Year	STDP	FHTET	NPS	VA. Tech	Total
2000	21,200	5,000	5,000	9,540	40,740
2001	21,728	5,000	5,000	9,778	41,506
2002	19,259			8,667	27,926
Total	$62,187	$10,000	$10,000	$27,984	$110,171

FY 2000 BUDGET REQUEST

Items	STDP	NPS	FHTET	VA Tech
Graduate Student Stipend @ 75%	10,643			
Student Wages	1,557			
Travel	5,000			
Materials and Supplies	4,000			
Indirect @45%				9,540
Total	$21,200	$5,000	$5,000	$9,540

STDP Production Function

Project Number: R8-2000-new project

Project Cost: $110,171

Project Objectives:
1. To develop a binomial sampling procedure that estimates hemlock woolly adelgid (HWA) density on a per tree basis. Such a protocol would be simpler than the currently used procedure requiring total counts on *x* number of twig sections.

2. Evaluate the role of tree and site measures as potential covariates for integration into the binomial sampling procedure.

3. Develop a website that calculates the population estimates for the user.

Assumptions:

For the purposes of this analysis the value of eastern hemlock as a timber species will be utilized. This value is only a small part of the overall importance of eastern hemlock to the forests and landscapes of the eastern United States. Eastern hemlock has numerous values that are not easily quantifiable. These values include aesthetics, recreational, ornamental, and ecological.

The development of simple sampling methods will enable resource managers to monitor the pest, estimate impact of natural enemies, and possibly develop hazard and risk ratings.
Developing a sampleing method will decrease survey and assessment time for all HWA projects by 75 percent.
Estimated loss of hemlocks due to currently inadequate sampling and survey methods is 50 percent.

The entire range of eastern hemlock will be infested within 30 years.

Estimated loss of hemlocks once infested is conservatively 50 percent.

Background Data:

Number of hemlock hectares is 1.3 million.

Approximately 325,000 hectares (25%) are currently infested.

Total cubic foot volume of hemlocks is 12 billion.

Total cubic feet of hemlock per hectare is 9230.7

Stumpage value per MCF of hemlocks is $46.87

Data extrapolated from USDA-FSD-FIA reports

Calculations:

EOV without project = (325,000 hectares)*(50% loss of trees)*(9.2MCF/hectare)*(46.87 per MCF) = 70,070,650 = discounted to 21,604,182

EOV with project = (325,000 hectares)*(50% loss of trees)*(9.2MCF/hectare)*($46.87 per MCF)*(1.2;20% probability of success) = 84,084,780 discounted to 25,925,019

Benefit Attributed to Project:

25,925,019-21,604,182 = $4,320,837

Benefit/Cost Ratio:

4,320,837/164,824 = 26.2

Benefit Attributed to STDP Funds:

26.2*$110,171STDP costs = $2,886,480

PNV of Project:

$4,320,837 - $110,171 = $4,210,666

PNV of STDP:

$4,320,837 - $62,187 = $4,258,650

LITERATURE CITED:

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