A Randomized Branch Sampling Method for Hemlock Woolly Adelgid

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ABSTRACT

An invasive exotic insect, the hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) has caused widespread mortality of eastern hemlock trees (*Tsuga canadensis* (L.) Carrière) and threatens to extirpate the species from North American forests. Neither HWA population densities nor their distribution in forests is well understood, hampering the ability of forest managers to respond to the pest. In addition, standard monitoring methods have inestimable bias and provide limited results. Lacking better information, land managers often have to assume that HWA is evenly distributed and saturates the environment. This paper explains a design unbiased sampling system appropriate to the biology of HWA and presents results of sampling HWA densities in New England forests.

KEYWORDS

Monitoring, population density, crown distribution.

INTRODUCTION

An invasive exotic insect, the hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) has caused widespread mortality of eastern hemlock trees (*Tsuga canadensis* (L.) Carrière) and threatens to extirpate the species from North American forests (McClure et al. 2001, Orwig et al. 2002). HWA-induced mortality is a concern because hemlocks provide important forest structure, habitat, economic benefits, and aesthetic values (Beatty 1984, Kelty 1989, DeGraaf et al. 1992, p. 92, Snyder et al. 2002). Unfortunately, lack of information about HWA impedes efforts to save forest hemlocks.

Information about the distribution of HWA within tree crowns and stands remains a gap in HWA research, in part because sampling methods for HWA are not well established. HWA is difficult to sample because of its small size, tree crown habitat, and lack of attractants. Current sampling for HWA often only includes the lowest portion of the crown adding inestimable bias to population estimates (McClure and Cheah 1999, Adams et al. 2002, Casagrande et al. 2002, Mayer et al. 2002). Before 2005 no studies had produced crown distribution estimates (Gray et al. 1998), although new research is underway (see Fidgen et al. and Costa in this volume). Thus far, population estimates have been general at best, and infesta-

tion levels are often just assumed from a decline in hemlock health (Bonneau et al. 1999, Orwig et al. 2002, Tingley et al. 2002). Without a good method to sample and monitor populations in the field, it is difficult to measure effectiveness of control efforts, catch new infestations, identify mortality risk factors, or even find resistant stands. This research details a sampling system designed for HWA and reports the results of monitoring HWA densities in New England forests.

METHODS

The goal of this study is to test randomized branch sampling (RBS) to estimate populations of HWA in a managed forest. The two main areas of interest are differences in HWA densities between heights within the crown and between nearby stands. Estimates of HWA populations within tree crowns may help future sampling efforts and estimates of differences between stands may aid HWA mitigation efforts. RBS is predicated on a design-based approach rather than a model-based approach to inference (Gregoire et al. 1995, Gregoire 1998). In other words, estimators generated from RBS of the mean, total, and variance require no assumptions about the HWA population sampled and are design unbiased (Gregoire et al. 1995). RBS treats a tree as a series of paths from the ground to each terminal shoot. Under RBS, the researcher randomly selects a path to a terminal shoot, and the characteristics of interest—the HWA and needles encountered along this path—become part of the sample. The RBS path can be terminated at any branching node to allow sampling of entire branches. The path is created by a series of random selections at each node. In other words, at each fork in the branch the researcher randomly chooses which branch to follow. The researcher can adjust the probability of selecting a branch to increase the likelihood of sampling more of the quantity of interest so long as the probabilities at any particular fork sum to one. No design bias is introduced if the selection remains probabilistic. The inverse of the product of the unconditional selection probabilities for a sample is used to inflate the sample to an estimate for the whole tree.

RBS provides an operationally efficient mechanism for unbiased estimation of the total, mean, and variance of the quantity sampled. Researchers have used RBS to estimate fruit production (Jessen 1955), tree weight (Valentine et al. 1984), total foliar area (Gregoire et al. 1995), stem length and surface area (Gregoire 1996), needle mass (Gaffrey and Saborowski 1999), tree biomass (Good et al. 2001), coarse woody debris (Gove et al. 2002), and floral distribution (Chen et al. 2003). This study is a new implementation of RBS to estimate insect populations.

I tailored the implementation of RBS in this study to the problem of estimating the number of HWA per needle in hemlock crowns. First, I fell the sample trees to permit access to the full crown. Felling damages the crowns, but comparisons of branches broken in the fall and all other branches shows no significant difference. Anecdotally, I have noticed that branch tips, which are the majority of the samples, are flexible enough to avoid damage in felling. After felling the sample tree, I stratify the crown into thirds and take at least three samples from each third.

This RBS scheme uses the simple random selection of the first node to more efficiently deal with the branching pattern of hemlock while retaining an unbiased design. I measure the diameter of all branches at each subsequent node in order to use the branch basal area as the selection probability of each branch. A field computer, a palm pilot with a custom application, records the selection probabilities, generates a pseudorandom number, and selects the branch to be included in the RBS path.

When a suitably small branch (<30cm in length) is selected, I clip it, place it in an envelope and return it to the lab for counting. I record the length of old and new growth, number of old and new needles, HWA ovisacs, sistens, and scale (*Fiorinia externa* Ferris and *Nuculaspis tsugae* Marlatt). In addition, I catalog stand, tree, and branch attributes such as stand basal area, tree height, crown height, diameter, branch direction, and branch height. These variables allow an investigation of the sources of variance in HWA within and between trees. Samples from the first nine trees included only counts of HWA ovisacs. The more recent 218 samples include the sistens generation of HWA on new growth as a more time-sensitive measure of HWA density, in addition to HWA ovisacs (Ward et al. 2004). All sisten counts are reported as the number of sistens per hundred needles so that the counts can be compared between branches of different health.

In 2004 I added ground-based sampling of the lower crown to the survey. These samples used a pole saw to select branches below 7.5m. I still selected branches using simple random selection and followed the RBS procedure after cutting them down so that estimates of the lower crown would be unbiased. In these samples the upper crown was not included in the sampling universe so unbiased estimators of the whole tree population are not available.

All sampled stands are hemlock or hemlock/hardwood mixtures about 80 to 100 years of age. Most stands are located on the Yale Myers forest in Union, Connecticut, within 5km of each other. I also sampled stands at Great Mountain Forest in Norfolk, Connecticut, and a forest in Sandisfield, Massachusetts. During this sampling effort I have counted over 300 branches on more than 80 trees over two seasons in 15 stands from three forests yielding nearly 140,000 needles and 7,000 adelgids.

RESULTS

In the fall of 2004, the population of HWA at Yale Myers Forest appeared to be much lower than it had been in 2003. The RBS for HWA documented a dramatic reduction in the estimated density of HWA in the three stands sampled in both 2003 and 2004. Figure 1 shows a graph of confidence intervals for three stands in both years. I used the mixed model procedure in SAS to analyze stand as a fixed effect, trees as a random effect, and branches as repeated measures (Littell et al. 1999, SAS Institute Inc. 2002). The mixed model deals appropriately with the correlation between trees in the same stand and constructs confidence limits for the least-squares means of the differences between stands. The unbiased estimators of total numbers of sistens per tree from RBS provide the data used in the mixed model of stand effect.

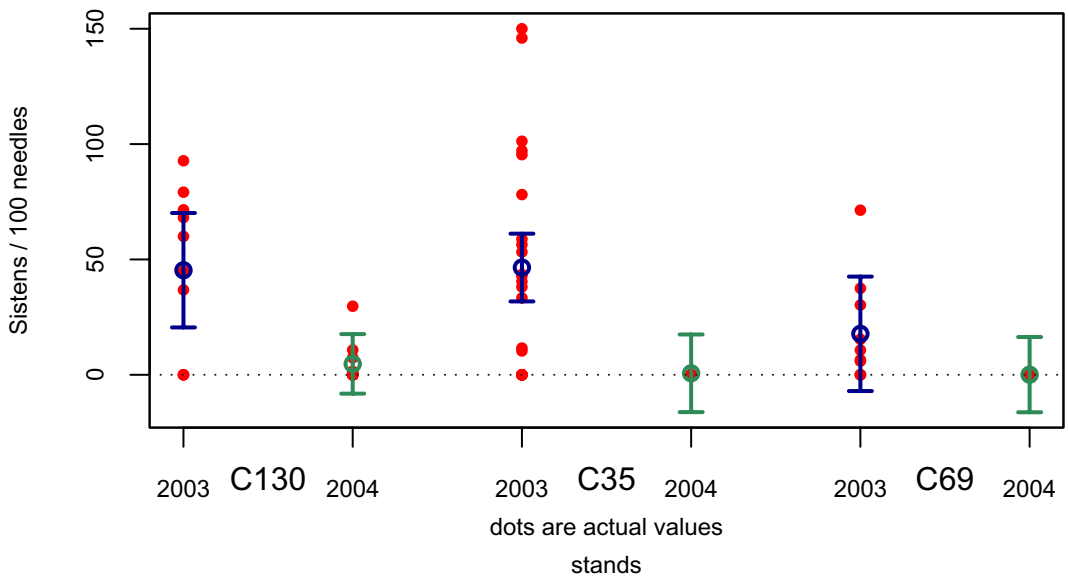

Figure 1. 90% confidence intervals for sistens per hundred needles in each of three stands for 2003 and 2004.

The differences between 2003 and 2004 are significant at the 90% level in both stands C35 and C130. Visual surveys and discussions with managers suggest that this reduction in densities is consistent across the forest.

In 2003 I sampled 174 branches from 16 trees in order to better understand how HWA is distributed throughout the crown. Figure 2 is a plot of the height at which the sample was taken versus the number of sistens per hundred needles recorded in that sample.

The plot of sistens versus sample height does not suggest any pattern. In the 2004 plot there is a cluster of values between 4 and 7.5m above the ground because many samples were collected with a pole saw that could only reach to 7.5m. I divided the crown in thirds and compared the number of sistens per 100 needles in each third in order to test for a pattern in branch height and HWA density (Figure 3).

Not only were there no significant differences between the crown strata estimates, but it seemed that further sampling might even show that the lowest crown stratum has more sistens per hundred needles than the top stratum. In part because of these results, I took fewer samples per tree and more samples across the stand during the second field season. Many samples were taken with a pruning saw and so can provide an unbiased estimate only for the first 7.5 meters of the crown. In addition the population reduction between 2003 and 2004 meant that many more samples were free of HWA. In some cases, sampled trees were almost completely free of infestation, and no RBS samples included sistens. On these trees, I made a purposeful sample of any sistens I could find to document the presence of infestation. Figure 4 includes purposeful samples as noted, although they are not included in the construction of confidence intervals.

Again there is no significant difference between the strata, although in 2004 the trend may be different from the previous year. I took too few samples from the middle and top

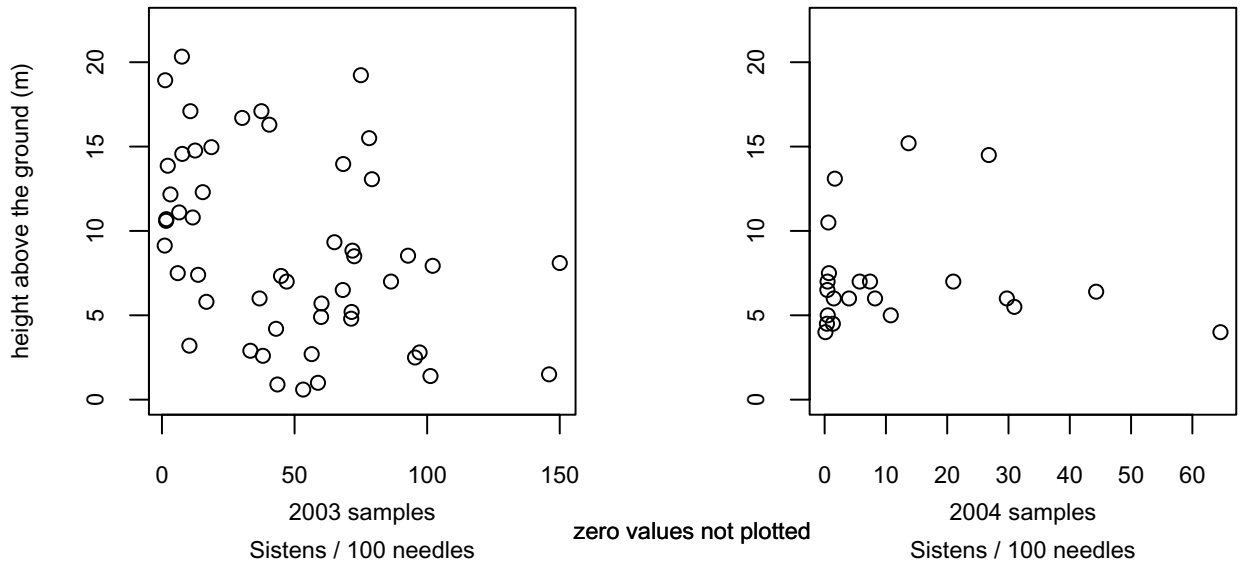

Figure 2. Height above the ground of each sample versus number of sistens per hundred needles.

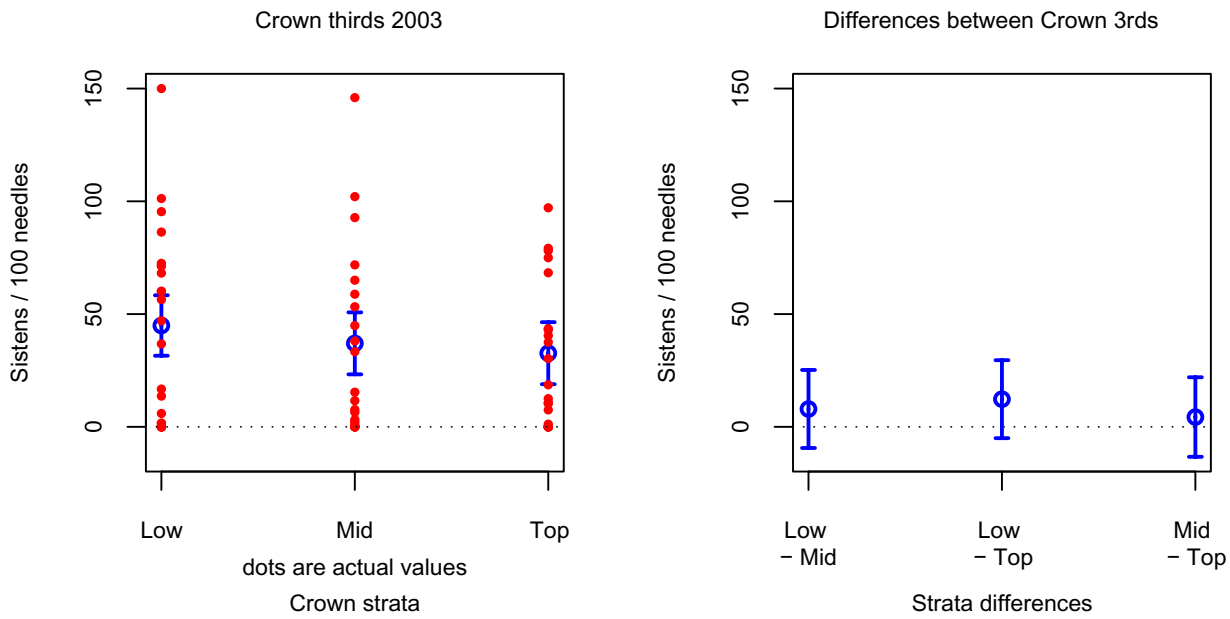

Figure 3. 90% confidence intervals for 2003 crown thirds and differences between crown thirds.

strata to be sure of any trends. Looking at the crown strata from all the 2004 samples together obscures the fact that many of the samples were only selected from the lowest 7.5m of the tree. One way to understand the potential bias in using only the lowest 7.5m is to investigate the number of samples with infestations in just the middle or top stratas. In 2003, 19% of the 16 infested trees sampled did not show infestations in the lowest portion of the crown. In 2004, none of the infestations were visible in the lower crown, although all three trees from

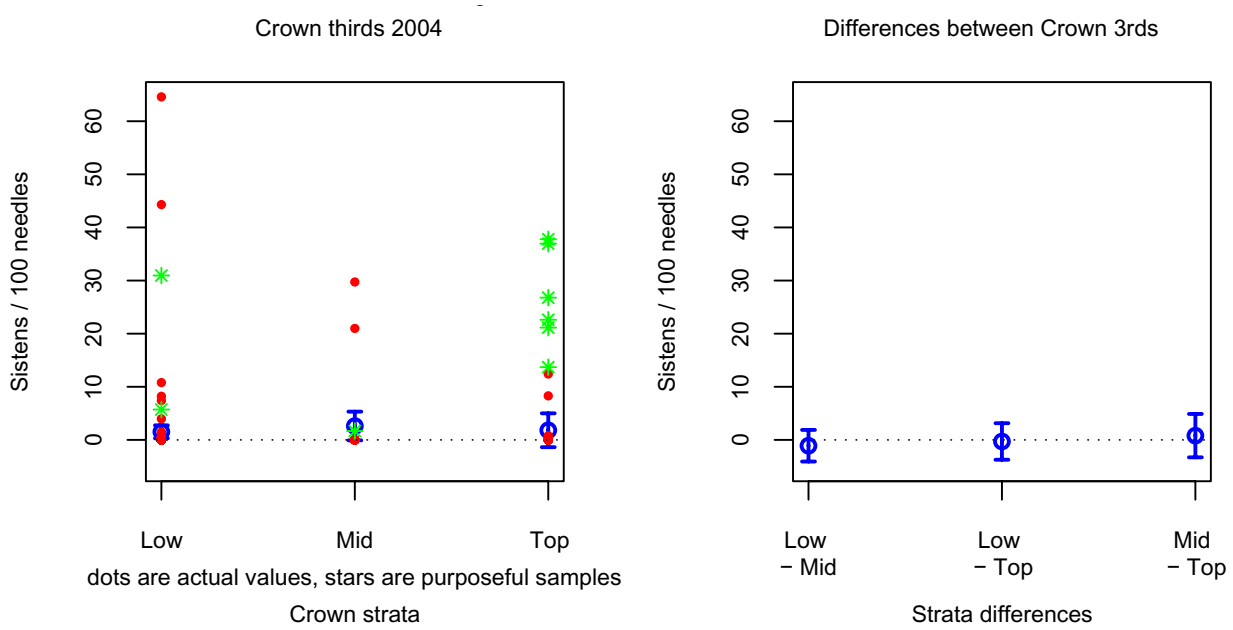

Figure 4. 90% confidence intervals for 2004 crown thirds and differences between crown thirds.

multi-strata samples were infested. A logging operation in stand C32B provided a second opportunity for understanding the bias of sampling only the lowest stratum. In stand C32B, I sampled the lower crown of six trees before logging and then, immediately after logging, I sampled six felled tops. The lower crown samples shown no infestations, but samples from the felled tops revealed very high densities of HWA.

Initial samples suggested there might be some differences between stands. In fact, analysis of HWA ovisacs per hundred needles showed stand C130 to be significantly different from the other stands at the 90% level. The sisten data for stands in 2003 does not show the significant difference that the ovisac data does because there are fewer samples per stand. Figure 5 graphs both ovisacs per hundred needles and sistens per hundred needles in the stands sampled in 2003. The sampling in 2003 focused on a greater number of samples per tree and fewer trees per stand, which made it hard to verify the existence of differences between stands.

I had hoped to be able to demonstrate differences between stands through a combination of branch samples from the lower crown and multi-strata samples. It would be possible to generate design unbiased estimates of the number of sistens per hundred needles in the stand by randomly selecting some trees for lower crown and some for multi-strata sampling. Unfortunately, in most stands I could only sample the lowest stratum because of the difficulty of safely felling trees. Therefore, I could only estimate the numbers of sistens on branches below 7.5 in these stands unbiasedly. Ironically, the bias of estimating the whole stand from lower crown samples may be much greater in 2004 than it would have been in 2003 because of the anecdotal evidence of differences between strata in 2004. Figure 6 depicts biased estimates of number of sistens per hundred needles in the stands based on pole saw samples but not purposeful samples.

The 2004 data shows no significant differences between stands, but this may be due to the bias of using lower crown samples. In addition, the low numbers of sistens in 2004 in comparison to 2003 make stand differences more difficult to pinpoint. At the lower number of sistens per tree in 2004, the sampling intensity would have to be increased to ensure detection of infestation. For example, tree 71 had a small HWA infestation on one branch 14.5m above the ground out of 112 branches, based on a visual census after felling.

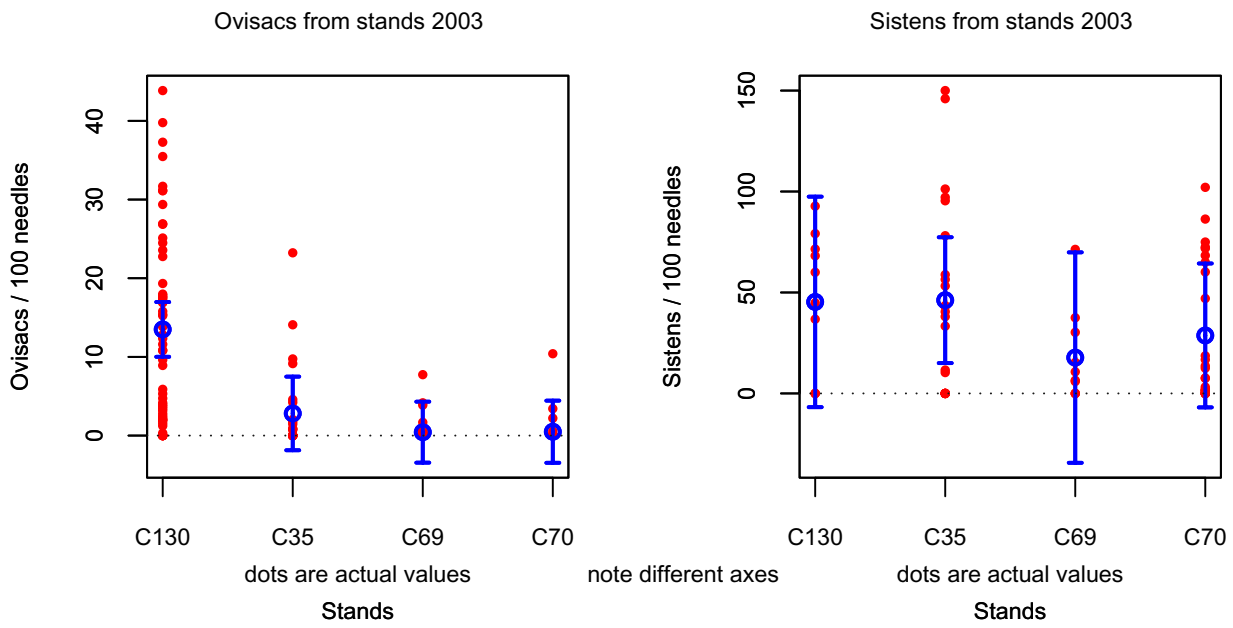

Figure 5. 90% confidence intervals for ovisacs and sistens from stands in 2003.

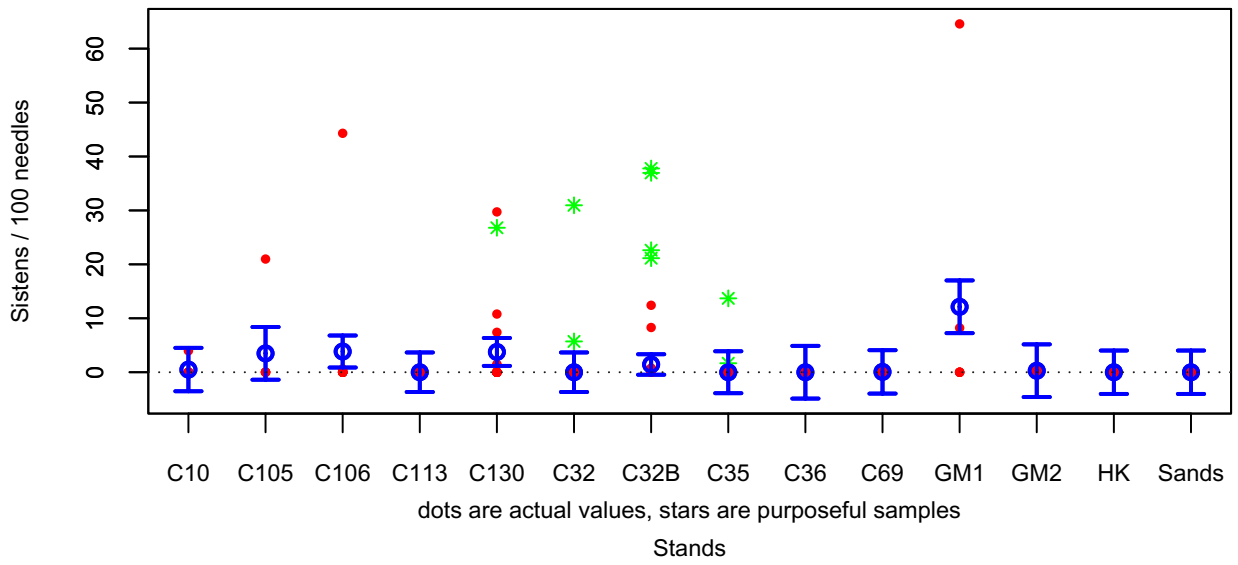

Figure 6. Biased 90% confidence intervals from stands in 2004.

CONCLUSIONS

This research shows the possibilities for using RBS to generate design unbiased estimators for an insect population that is difficult to monitor. The RBS survey at Yale Myers Forest shows large decline in the HWA population in the three stands sampled. Although populations declined, infestations were still present. 2003 data shows some differences in numbers of HWA ovisacs between nearby stands, while data from 2004 is inconclusive. The number of HWA ovisacs per hundred needles was significantly different in C130 from C69 and C70 in 2003. In 2003, there was no significant difference between crown strata. Anecdotal evidence from sampling in 2004 suggests higher HWA populations in the upper crown than the lower crown. It may be that, at high population densities, the difference between upper and lower crown strata is less than the difference between crown strata when HWA densities are low. Further sampling from all crown strata is necessary to better understand the densities of HWA throughout the crown.

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