

# Operation Adelgification: Evaluating a Rain Down Technique to Artificially Infest Seedlings with the Hemlock Woolly Adelgid

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## INTRODUCTION

Over the last two decades, the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand) has caused widespread mortality among populations of Eastern (*Tsuga canadensis* (L.) Carrière) and Carolina (*Tsuga caroliniana* Engelman) hemlock throughout the eastern United States. The integrated strategy to manage the impacts of this invasive insect pest on eastern forests includes biological control, chemical insecticides, silvicultural practices, and breeding for host resistance. Since 2002, considerable progress has also been made in understanding how some hemlocks are able to resist adelgid attack through research that has focused on genetic conservation and diversity (Potter et al. 2008; Jetton et al. 2010) and the influence of hemlock species and foliar chemistries on susceptibility to adelgid infestation (Lagalante and Montgomery 2003; del Tredici and Kitajima 2004; Pontius et al. 2006; Evans 2008; Jetton et al. 2008; Kaur et al. 2008; ; Hoover et al. 2009; Ingwell et al. 2009; Weston and Harper 2009). Putatively HWA resistant Eastern hemlocks have been identified and propagated for further study (Caswell et al. 2008), and the potential for increased levels of tolerance to adelgid infestation in Carolina hemlock has been cited (Jetton et al. 2008; Kaur et al. 2008). Finally, attempts to create interspecific hybrids between adelgid resistant *T. chinensis* (Franch.) E. Pritz and the generally susceptible species *T. caroliniana* and *T. sieboldii* Carrière have been successful, and hybrids have been found to have levels of HWA resistance intermediate between the pure species (Bentz et al. 2002; Pooler et al. 2002; Montgomery et al. 2009).

To build upon this current understanding of adelgid host resistance in hemlock, the development of artificial infestation techniques that are efficient and reliable for screening large numbers putatively adelgid resistant genotypes has been identified as a key research priority by the USDA Forest Service Working Group on Genetics and Host Resistance in Hemlock. Here we report on a pilot study designed to address this topic and to test the distribution and abundance patterns of first instar HWA crawlers in a rain down technique originally developed to artificially infest fir (*Abies* spp.) seedlings with the balsam woolly adelgid (*Adelges piceae* Ratzburg) (Newton et al. 2007).

## METHODS

- Eastern hemlock branches infested with HWA sistens adults and progrediens eggs were collected on 18 March 2011 from non-declining trees. About 300 branch tips, 40 cm long were cut from this material. Mean (SE) HWA densities on a sample of 15 branches were 216.6 (93.3) ovisacs/branch and 45.7 (5.8) eggs/ovisac.
- On 22 Mar 2011, infested branch tips were placed bottom-side-down on top of cubic frames (1m x 1m x 1m) constructed of 2-cm diameter PVC pipe, covered on the top with poultry wire and on the four vertical sides with plastic sheeting (Fig 1). Frames were placed over a 1 m<sup>2</sup> sheet of paper printed with a 10 x 10 cm grid (100 squares) and covered with a thin layer of insect glue.
- Four frames received a “low” ovisacs density treatment (24 branch tips) and 4 frames received a “high” ovisac density treatment (48 branch tips). A Carolina hemlock seedling was placed beneath the frame on one half of the glue sheet. An eastern hemlock seedling (unavailable at the beginning of the experiment) was added next to each Carolina hemlock seedling after 9 days. Crawlers were allowed to “rain down” on glue sheets and seedlings for 3 weeks (Fig. 2). Seedlings were watered weekly.
- After 3 weeks (12 Apr 2011), seedlings and the 1 m<sup>2</sup> gridded glue sheets were removed from the frames. The number of progrediens crawlers raining down per unit area was estimated using a subsampling scheme. A 60 x 100 cm section of the 1m<sup>2</sup> glue sheet (that which was not covered by seedlings) was divided into fifteen 20x20 cm sheets (Fig. 3). Four 2x2 cm squares were randomly selected from each of the 20x20 cm sheets, and the number of progrediens crawlers in each square was counted. Thus counts were made on a total of 60 2x2 cm squares per frame.
- The number of settled progrediens on the Carolina and eastern hemlock seedlings will be estimated from ovisac counts in May 2011.



Fig. 1. Cubic (1m<sup>3</sup>) PVC frame used in the adelgification experiment. A total of 8 cubic frames were used, 4 per ovisac density treatment.



Fig. 2. After 1 week there was visible evidence of HWA crawler “rain down” inside the PVC frames. Based on visual assessment, crawlers were evenly distributed across the 1m<sup>2</sup> glue sheet, but there were “hot spots” where crawler density was particularly high.

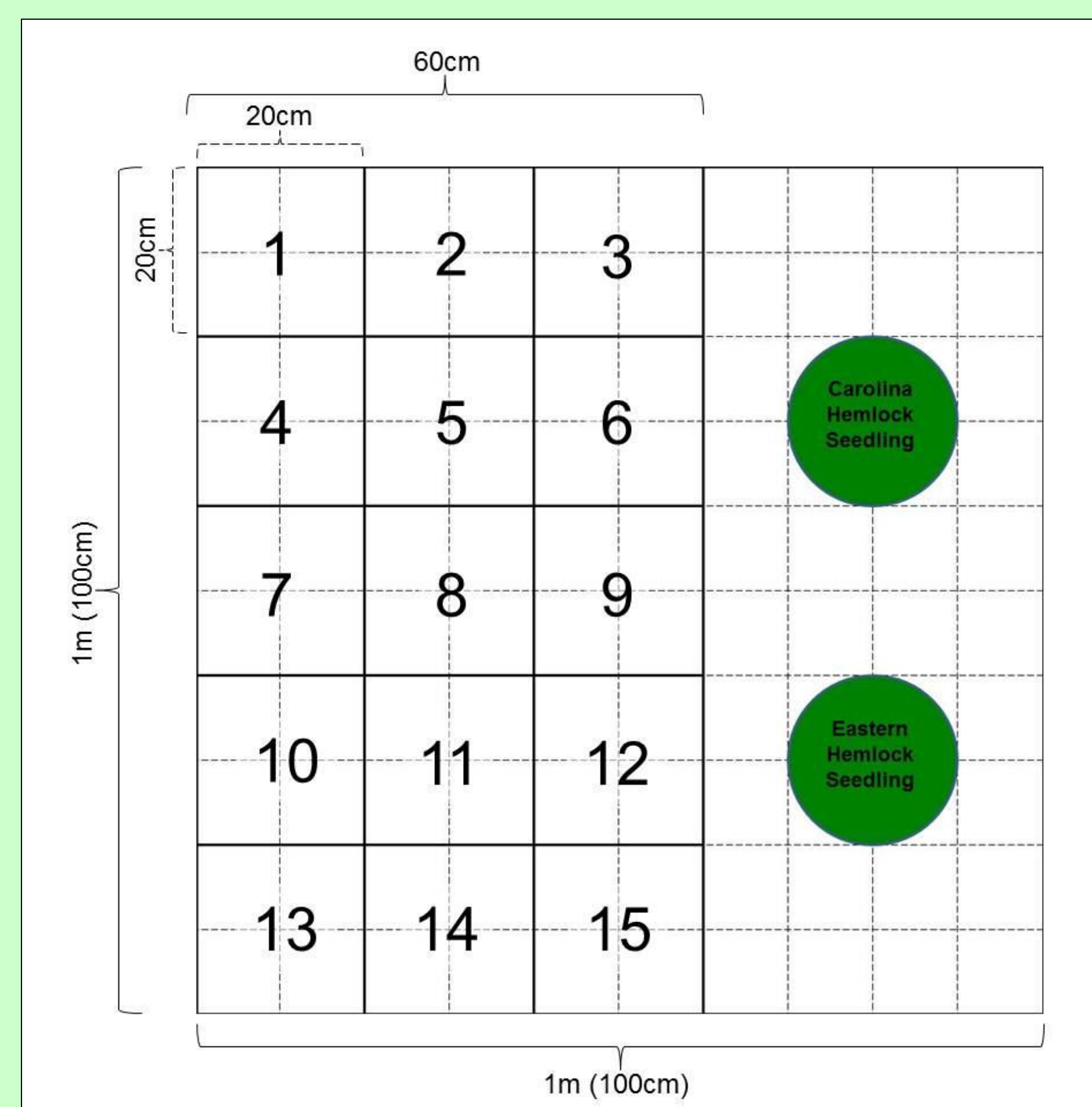


Fig. 3. Sampling plot layout within each PVC frame. Within each of the fifteen 20x20cm subplots, four 2x2cm squares were randomly selected for crawler counts.

## RESULTS

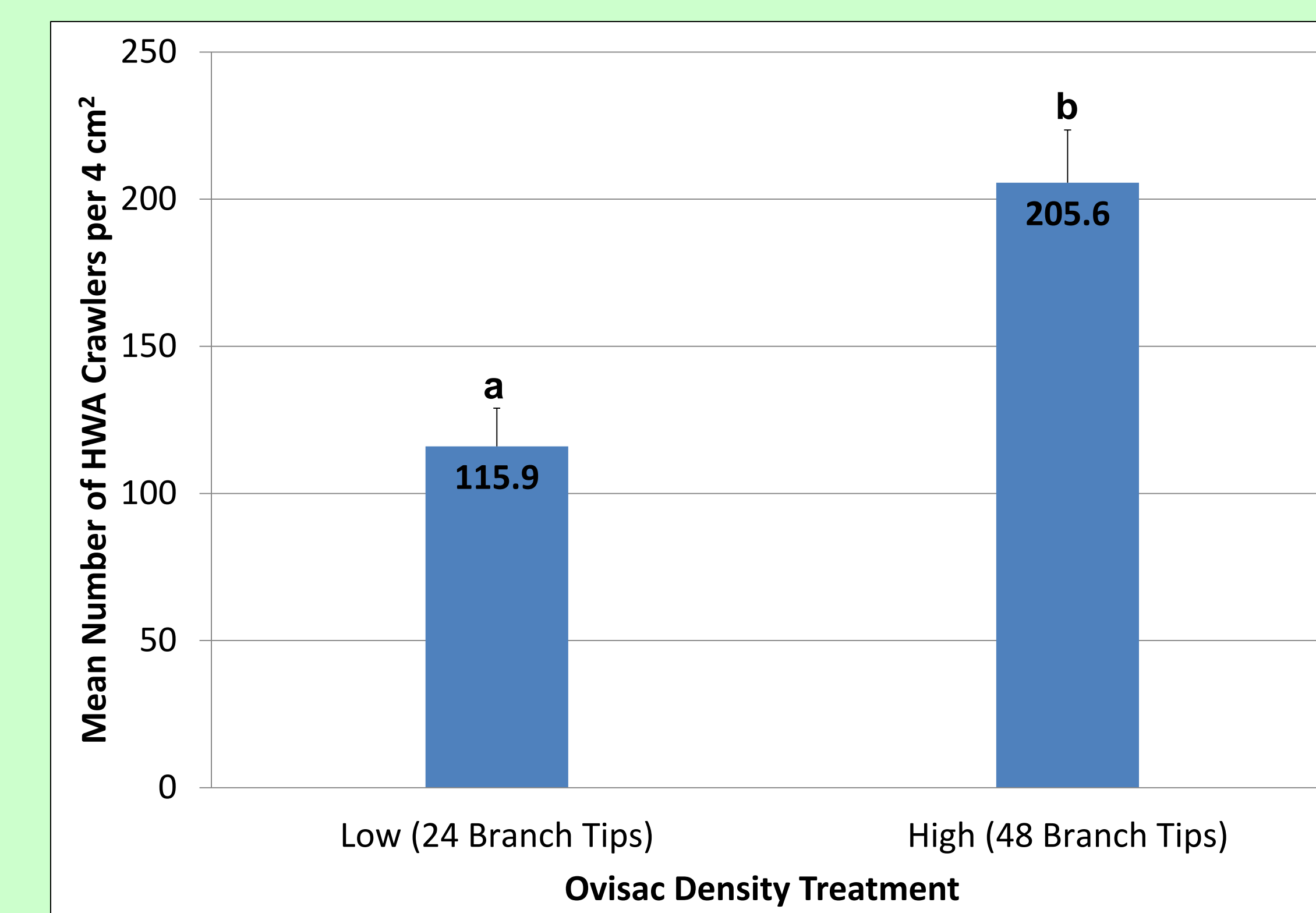


Fig. 4. Mean (±SE) number of HWA progrediens crawlers per 2x2cm sample that “rained down” on glue sheets in each ovisac density treatment. Columns with different letters are significantly different at  $\alpha \leq 0.05$ .

a)				b)			
51.68 (± 14.42)	79.85 (± 10.79)	59.06 (± 7.33)		137.93 (± 26.17)	210.68 (± 76.79)	447.31 (± 146.31)	
53.56 (± 7.77)	106.62 (± 24.39)	182.43 (± 40.19)	Carolina Hemlock Seedling	87.93 (± 13.88)	227.75 (± 67.94)	291.50 (± 89.88)	Carolina Hemlock Seedling
79.12 (± 4.03)	62.43 (± 11.70)	201.18 (± 85.07)		107.18 (± 23.60)	150.25 (± 11.32)	252.12 (± 70.91)	
50.18 (± 13.29)	304.68 (± 128.79)	286.50 (± 86.87)	Eastern Hemlock Seedling	144.31 (± 54.34)	342.06 (± 100.36)	149.31 (± 20.33)	Eastern Hemlock Seedling
67.25 (± 18.35)	100.68 (± 20.38)	54.31 (± 4.14)		92.12 (± 13.91)	299.87 (± 90.91)	143.43 (± 41.12)	

Fig. 5. The spatial distribution (mean number of crawlers ±SE) of HWA progrediens crawlers among the fifteen 20x20cm subplots in the low (a) and high (b) ovisac density treatments.

## DISCUSSION and CONCLUSIONS

- After evenly covering a raised 1m<sup>2</sup> wire frame with HWA-infested branches, we found that progrediens crawlers “rained down” and were well distributed over the surface below. Occasional “hot spots” of highly-concentrated crawlers on the glue sheet presumably resulted from corresponding dense clusters of ovisacs above.
- The “high” ovisac treatment (48 branches) resulted in significantly more crawlers raining down than the “low” ovisac treatment (24 branches) ( $t = 2.748$ ,  $df = 6$ ,  $P \leq 0.0001$ ). Extrapolating from mean counts made on 2x2 cm squares, an estimated 290,000 and 514,000 crawlers per m<sup>2</sup> fell beneath the low and high ovisac treatments, respectively.
- This rain down technique shows promise for applying ample infestation pressure on large numbers of seedlings simultaneously in resistance screening trials. Current and future work will compare infestation success on seedlings subject to the rain down technique versus other methods of adelgification.

## ACKNOWLEDGMENTS

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