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Density-dependent Survival of Hemlock Woolly Adelgid Life Stages

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**DENSITY-DEPENDENT SURVIVAL OF HEMLOCK WOOLLY ADELGID
LIFE STAGES**

A Thesis Presented

by

ELIZABETH M. SUSSKY

Submitted to the Graduate School of the University of Massachusetts Amherst in partial
fulfillment of the requirements for the degree of

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DEDICATION

For my father, Michael.

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ABSTRACT

DENSITY-DEPENDENT SURVIVAL AND FECUNDITY DECLINE OF HEMLOCK WOOLLY ADELGID IN A MASSACHUSETTS FOREST

SEPTEMBER 2013

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To investigate the density-dependent factors that may be partly responsible for the apparent stability of hemlock woolly adelgid (HWA; *Adelges tsugae* Annand) populations in central New England, we infested 64 eastern hemlock (*Tsuga canadensis* Carrière) trees with varying densities of HWA sistens ovisacs in a typical eastern hemlock forest in western Massachusetts. We subsequently documented HWA density, fecundity, and the amount of new growth on experimental trees over two consecutive years. We used a 2 x 2 randomized block design using previously and newly infested hemlocks divided into 1 m tall saplings and branches of mature trees. There was a density-dependent decline in the survival and fecundity of HWA in both the spring and winter generations. This response was a function of both previous infestation by HWA and current years crawler density in the spring generation. Additionally, the production of sexuparae in the spring generation played a key role in the overall density-dependent survival of HWA, suggesting that sexuparae production is strongly linked to developing crawler density. In Chapter 2, varying densities of HWA were manipulated on 16 previously uninfested eastern hemlocks in an open field plantation. In contrast to HWA

populations in our forest experiment, there was no evidence of density-dependent survival on a tree-wide basis in the plantation in the springtime progrediens generation. However, there were comparable density-dependent survival of settled crawlers and sexuparae production when samples of the population were examined from branches with high density. Plantation hemlocks had 9.3 times more foliage and ten times lower HWA densities per cm than forest hemlocks. Despite the lack of density-dependence in the progrediens generation of this study, HWA populations of the subsequent sistens generation went extinct on 13 out of the 16 trees. These results show that density-dependent processes may only exist when HWA density/cm reaches a certain threshold, and that high mortality may occur during the late-summer aestivation phase at densities below this threshold, perhaps due to high summertime temperatures in HWA's sistens generation. These results may help explain the slow process of HWA establishment in our region.

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CHAPTER 1

DENSITY-DEPENDENT SURVIVAL AND FECUNDITY DECLINE OF HEMLOCK WOOLLY ADELGID IN A MASSACHUSETTS FOREST

1.1 Introduction

In North America, invasions by non-native forest insects increasingly threaten the stability of native forest ecosystems through the destruction of dominant tree species (Liebhold et al. 1995, Orwig 2002, Lovett et al. 2006). Host trees attacked by non-native forest insects with which they lack a co-evolutionary history are often extremely vulnerable to the effects of the invasive insect (Morin et al. 2009, Gandhi and Herms 2010). In addition to host vulnerability, newly invaded environments often lack sufficiently specialized natural enemies, ones able to prevent invasive populations from reaching outbreak densities on their host.

In the eastern United States, the effects of hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) (hereafter HWA), an invasive forest insect from Asia (Fig. 1A), has been a prime example of host vulnerability to a novel insect. The HWA has decimated many stands of a dominant tree species, either eastern hemlock (*Tsuga canadensis* Carrière) or Carolina hemlock (*T. caroliniana* Engelmann), since its initial introduction to Virginia in the 1950's (Orwig 2002). Hemlock woolly adelgids are transported by wind, wildlife and humans (McClure 1990), and over the last 60 years, populations have spread south to Georgia and north to southern Vermont, New Hampshire and Maine (Orwig and Foster 1998, Preston 2007). Damage from HWA has been most severe in the southern and mid-Atlantic states, where irreversible hemlock decline has occurred in as little as four years (Eschtruth 2006). This has led to major species shifts in hemlock dominated ecosystems (Jenkins et al. 1999, Orwig et al. 2002,

Kizlinski et al. 2002, Evans 2004, Stadler et al. 2005, Eschtruth 2006, USDA 2012). Damage from HWA has slowed substantially, however, in its northern range. After 20 years of HWA infestation in Massachusetts, few stands have experienced significant hemlock mortality and HWA populations appear to have stabilized (Paradis 2011, Orwig et al. 2012). Colder winter temperature in the north which cause greater overwintering mortality of HWA has been accepted as the likely explanation for this lack of hemlock mortality in northern stands infested with HWA (Shields and Cheah 2005, Trotter and Shields 2009). Paradis (2011), however, demonstrated that HWA mortality in Massachusetts is consistently higher in the late-spring and summer months compared to the mortality occurring in the winter. This suggests that overwintering mortality may not exclusively explain the observed stabilization of northern HWA populations. We need to understand the various causes of mortality or changes in fecundity that may stabilize HWA populations at various stages in the life cycle; as such processes may explain why hemlocks in Massachusetts have persisted despite the invasions by HWA.

Population densities of all herbivores are regulated or stabilized either by mortality caused by natural enemies or by interaction with their host plants (Murdoch 1994). HWA lack parasitoids (Cheah et al. 2004), and in the eastern United States native predators exert little impact on HWA populations (Montgomery and Lyons 1996). Predatory beetles have been released in New England but have not yet established in significant numbers (Paradis 2011).

Previous observations of naturally occurring HWA-infested hemlocks in Connecticut and Massachusetts by McClure (1991) and Paradis (2011) provided evidence of HWA population regulation via density-dependence in the springtime progrediens generation.

McClure's (1991) study of HWA population dynamics revealed a 2-year boom and bust cycle, in which a rise in HWA density produced a decline in the health of the hemlock host and a cessation of new shoot growth the following year. The host response (i.e., the lack of new shoot growth) caused a decline in HWA density the following year, whereupon the hemlocks recovered, produced new growth and HWA populations subsequently increased. The hemlocks declined a second time in the fourth year, and in McClure's study, all trees died. Paradis (2011) collected data similar to McClure's at six sites across New England. In this study, HWA density fluctuations were similar to those documented by McClure (1991), but even when HWA densities were at their highest levels, the hemlocks produced some new growth, and the HWA/hemlock cycles persisted for the six years of her study without any tree mortality. These observations matched the general pattern often observed in Massachusetts, where most HWA infested hemlocks remain alive for years (Preisser et al. 2008, Paradis 2011, and Orwig 2012).

Both McClure (1991) and Paradis (2011) documented strong negative density-dependent survival in the progrediens generation. McClure (1991) showed that much of this mortality was caused by density-dependent sexuparae production, which is suicidal for HWA in North America, as explained below. Paradis (2011) showed that HWA progrediens fecundity is also negatively density-dependent. Simulations by Elkinton et al. (2012) showed that these factors alone could account for most of the observed dynamics in the HWA population system, i.e., the boom and bust cycle. These simulations also showed that this density-dependent mortality strongly compensates for any predation on the eggs produced by the sistens generation in early spring. On the other hand, if sexuparae production is determined by the parent when the eggs are laid, then this

density-dependent mortality precedes rather than follows egg predation. These considerations made it clear that there is a need to understand the cause and timing of mortality in the progredien stage.

The objective of our study was to quantify each component of HWA survival in both the progredien and sisten generations in relation to HWA density, tree health, and tree age. We aimed to understand what proportion of the density-dependent survival in each stage was caused by the previous effects of HWA on the tree and how much was due to a direct response to current year HWA density. We inoculated previously infested and uninfested sapling and mature eastern hemlocks with HWA ovisacs produced by overwintering sistens at varying densities. Our findings improve the understanding of the timing and causes of density change and population regulation of HWA.

1.2 Methods

1.2.1 The study system

HWA feed on the ray parenchyma cells of hemlocks by inserting their stylets at the base of needles (Young et al. 1995). As HWA develop, a white protective wool-like wax is secreted from the female to which it will eventually oviposit within (McClure 1989, 1990, 1991, Fig. 1.1A). HWA has two parthenogenic generations per year. The overwintering sistens generation lays eggs that hatch in April and May, producing the springtime progrediens generation (McClure 1989). Some progredientes become winged sexuparae (Fig. 1.1B) that in its native Japan, disperse to spruce (*Picea* spp.) and initiate a sexually reproducing generation.



Figure 1.1. (A) Heavily adelgid infested hemlock twig. As the adelgid develops, it secretes a white protective wool-like wax within which it will oviposit; (B) newly emerged sexuparae. Photos from (A) Connecticut Agricultural Experiment Station and (B) E.M.S. 6/7/12

In North America, however, none of the offspring of these sexuparae survive on the native spruce species, so there is no sexual life cycle and only parthenogenic reproduction occurs (McClure 1989). The asexual progredien crawlers settle on hemlock shoots produced in the previous year. They mature and oviposit in June, and eggs hatch in late June or July. The resulting sisten crawlers settle preferentially on current year shoots, and enter a period of summer aestivation, where feeding and development cease. Feeding and development resumes in October, and continues throughout winter. In the northeastern United States, the sistentes oviposit in March, and eggs hatch in late spring (Fig. 1.2).

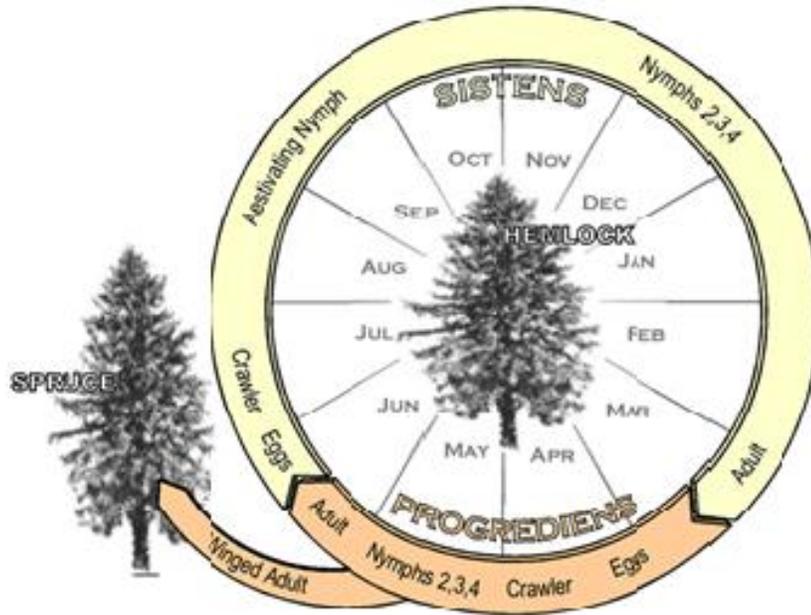


Figure 1.2. Hemlock woolly adelgid annual life cycle on hemlock in North America. Photo from Cheah *et al.* (2004)

In 1995, HWA was first observed at our study site (Orwig and Foster 1998), a mixed hemlock-hardwood forest near the Quabbin Reservoir in Pelham, Massachusetts. Our experiment was the second of two done with HWA populations at the site. The first was a study of the effects of HWA on hemlock foliage chemistry by Pezet *et al.* (in press). When our experiment began in 2011, almost none of the trees in our experimental stand were naturally infested with HWA and we removed any that were found.

1.2.2 Experimental design

In late-spring of 2011 and 2012, just before progredien hatch, eastern hemlock branches approximately 30 cm in length were collected as experimental inoculum. These branches were collected from nearby forests, and were heavily infested with healthy HWA ovisacs. Inoculum branches were grouped in bundles of 1, 3, 10, or 30, placed in

water-saturated floral foam, and attached to experimental hemlocks at the study site the following day.

Experimental units consisted of either 32 hemlock saplings or 32 lower branches of mature hemlocks. In 2011, half of the trees or branches had been inoculated the previous year by Pezet *et al.* (in press), but there was no subsequent survival of HWA due in part to extreme winter temperatures and consequently very high overwintering mortality. In 2012, the newly infested trees from 2011 were considered previously infested. Any surviving HWA were removed as we set up the experiment. The four experimental treatments were grouped by physical proximity into sixteen blocks to create a randomized block design. Four different density levels (with 1, 3, 10, or 30 inoculum twigs) were created and randomly assigned to experimental trees. Inoculum twigs were spaced out across the experimental trees or branches and left in place for three weeks to ensure colonization of branches by newly emerged progredien crawlers.

1.2.3 Sampling method

After successful establishment of the progredien crawlers, the twigs used as inoculum for infestation of experimental field hemlocks were removed and brought to the laboratory to measure density and fecundity of the sistens generation. Fecundity of the sistens adults was determined by counting the average number of chorions from hatched eggs in three woolly egg masses on each bundle of inoculum twigs. The number of progredien crawlers produced on each experimental branch or tree was estimated by multiplying the number of previous generation sistens on the inoculum branches by mean fecundity estimated from all trees in the experiment. In this way, the four density classes were converted into a continuous variable of inoculation density of progrediens crawlers.

To estimate sexuparae production in the spring generation, shortly following the establishment of spring generation crawlers, sample twigs were cut from each inoculated tree and placed in water picks. The twigs were held in the laboratory in small Berlese funnels (BioQuip Products, Rancho Dominguez, and CA) to collect emerging sexuparae. The cut ends of the twigs were placed in 30 ml water filled jars sealed with parafilm (Cole-Parmer, Vernon Hills, IL) which served to keep the hemlock foliage fresh and the HWA alive for several weeks. Settled progrediens nymphs on these twigs were counted under the microscope and the survival and fecundity of those adelgid that lived long enough to secrete wool and reproduce was determined. The number of progredientes that reached maturity was also recorded for each experimental hemlock in the field.

There are three components to survival in the springtime progredien stage: survival of crawlers not dispersing (S_{disp}); the proportion of those crawlers becoming progredientes and not sexuparae (S_{sex}); and survival of settled progredientes (S_{set}). The total survival in the progredien generations (S_{prg}) is the product of these three components. Although we did not measure survival from dispersal directly, we equate it with the proportion not dispersing and can estimate it as $S_{disp} = S_{prg} / (S_{set} * S_{sex})$. We recognize that this estimate does not include the unknown and probably small proportion of individuals that disperse from the sapling tree or mature branch, but settle successfully on new hosts.

In early August when it was certain that all of the sistentes had settled, a branch from each experimental tree or branch was haphazardly selected and the terminal 30 cm were marked off with flagging tape. On each branch terminal, the proportion of twigs with new growth, the total length of new growth and the number of settled sistentes were recorded.

Aestivation survival was determined by counting the number of sistentes that survived to produce wool by December on both the 30 cm sample branches and on the entire experimental tree or branches. Pre-winter sistens survival for the entire pre-winter period was estimated by the ratio of sistentes that had put on wool by December to the estimated number of sisten crawlers produced (progre dien adults * progre diens fecundity).

For each experimental tree or branch, the length and width of each limb on saplings and branches of mature trees, as well as the total twig and branch length (sum of lengths of all needle bearing twigs) on 10 saplings and 10 mature branches were measured.

Plotting these two measures against each other yielded the following equation: $y = 0.369x - 51.235$, ($R^2 = 0.8058$, Figure 1.3), which we used to obtain an estimate of total twig length on the entire tree from length x width measurements of all branches in the 1 m tall saplings and from all major branchlets that made up the sample branches from the mature trees. The total length of hemlock twigs is proportional to branch area, because twigs elongate across both the width and length of the branch as it grows.

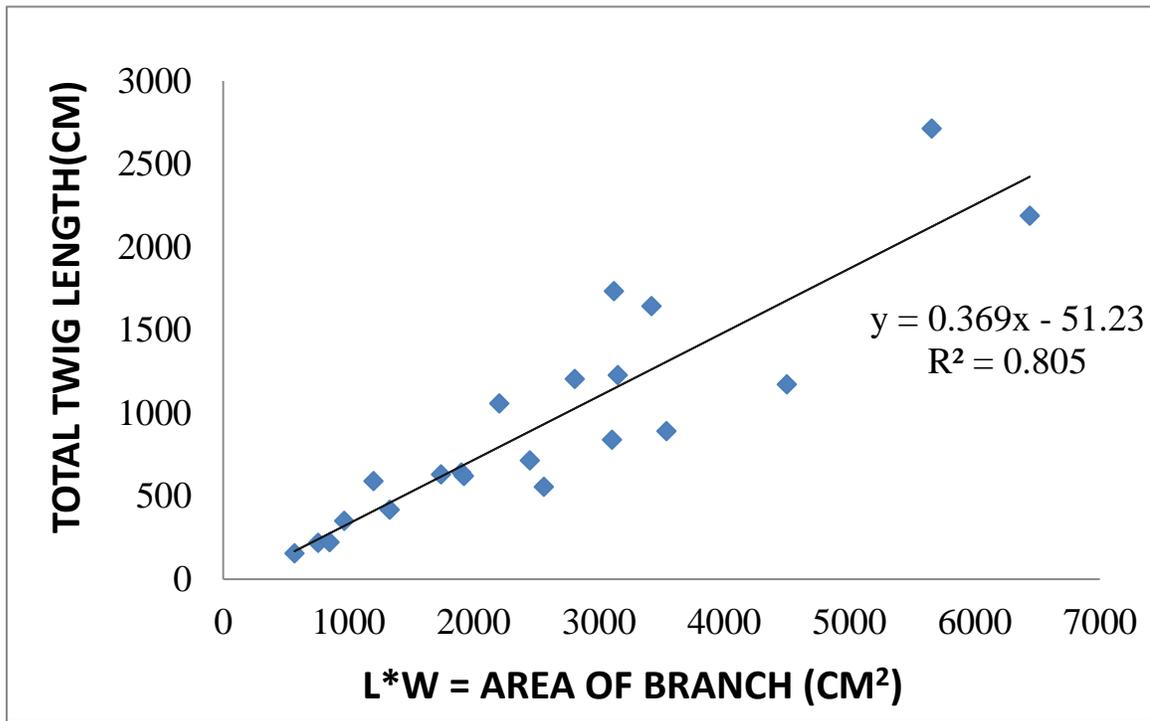


Figure 1.3. Linear regression obtained from branch length x width measurements and total twig length per branch on hemlocks at the forested site.

1.2.4 Data analysis

We analyzed data using a mixed model ANCOVA (PROC Mixed) in SAS 9.3 (SAS Institute 2012), with two fixed effects: previously infested versus newly infested trees and saplings versus branches of mature trees, and HWA density ($\log_{10}(\text{HWA}/\text{cm})$) as a covariate. Blocks were included as a random variable. The dependent variables in these analyses were \log_{10} (proportion surviving) of different HWA life stages, progreddiens fecundity or mean length of new growth of hemlocks. All non-significant interactions were removed from models in final analyses (Tables 1.1-1.4). This analysis allowed us to determine the effects of our covariate, HWA density, on subsequent HWA survival, fecundity, and hemlock new growth throughout various life stages of HWA and in response to our two treatments: previous infestation and tree age.

1.3 Results

In both years, we observed a pronounced density-dependent decline in survival of the entire springtime generation (Figs. 1.4 and 1.5). Survival to the adult progredien stage was always less than 10% (log survival < -1 , Figs. 1.4A and 1.5A), and on most trees it was less than 1% (log survival < -2). Total survival in this stage included density-dependent survival of settled progredientes (Fig. 1.4B and 1.5B), survival from sexuparae production (Fig. 1.5C), survival from sexuparae production after the crawlers had settled (Fig. 1.5D), as well as the proportion of those that did not disperse (Figs. 1.4C). We also observed a strong density-dependent decline in progredien fecundity (Figs. 1.4D and 1.5F). In 2012, we observed substantial sexuparae production, beginning from early June through mid-July, and averaging 65% across all treatments. We observed no sexuparae production in the identical experiment the previous year.

Consistent between the two years, infestation by HWA the previous year produced the expected decline in the proportion (Figs. 1.4E and 1.5G) and length (Figs. 1.4F and 1.5H) of new growth on experimental hemlocks. This decline in new growth, however, was not related to the overall survival of the current generation (Figs. 1.4 E and 1.5G), or the current year density of inoculated progredientes (Figs. 1.4F and 1.5H).

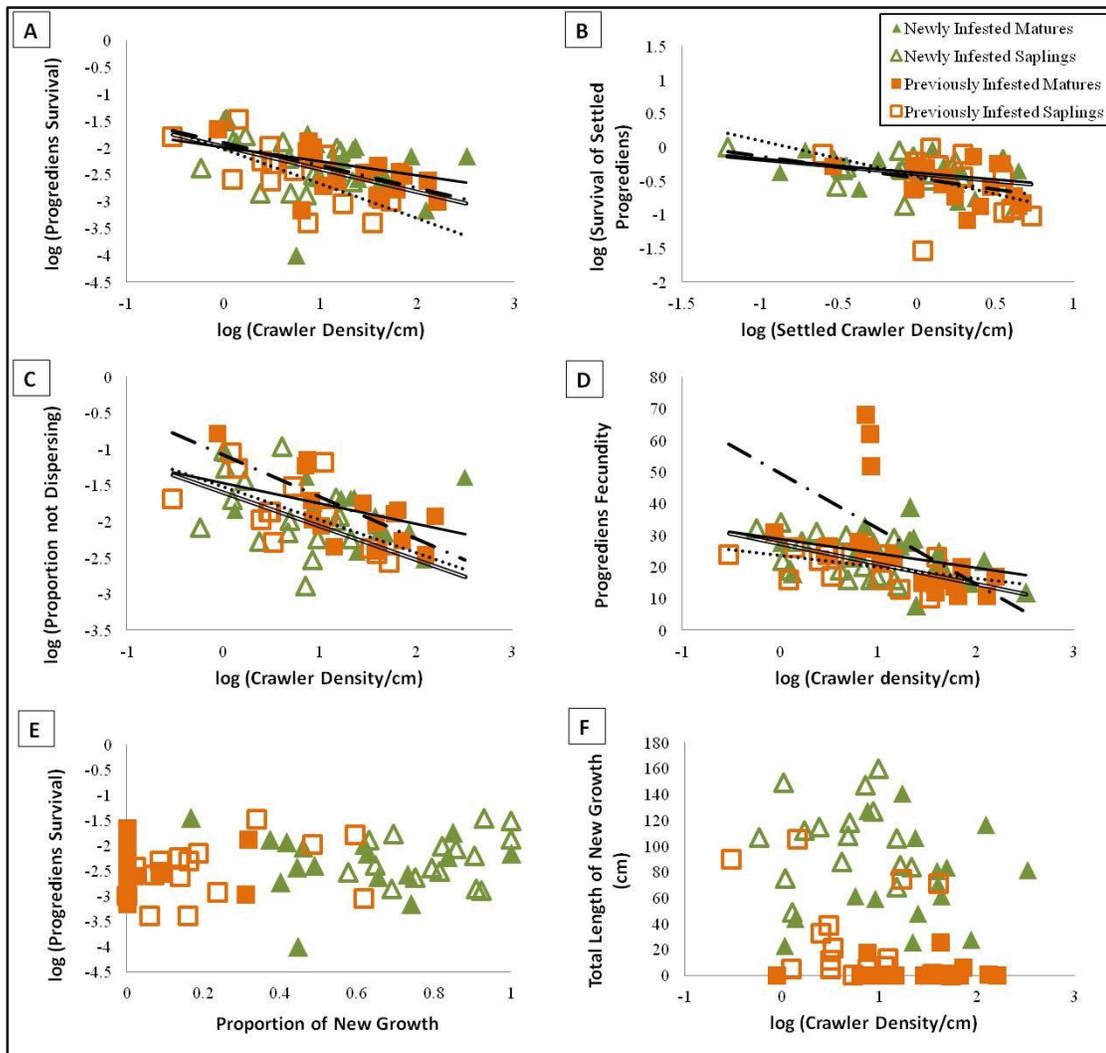


Figure 1.4. HWA density, fecundity, survival, and new hemlock growth during the spring generation in 2011. (A) Total density-dependent survival in the springtime progrediens generation; (B) Density-dependent survival of settled progrediens crawlers; (C) Density-dependent dispersal survival; (D) Density-dependent fecundity decline in the progrediens generation; (E) Proportion of new growth on progrediens survival; (F) progredien crawler density on amount of new growth (cm). Regression lines only plotted for significant covariate effects; — Newly Infested Mature, — Newly Infested Saplings — · Previously Infested Matures, ····· Previously Infested Sapling Hemlocks.

Table 1.1. Statistical analyses¹ of HWA survival in the springtime progrediens generation from experiments performed in Massachusetts in 2011.

Fig 1.4	Dependent variable	Covariate	Sapling vs. Mature df=1		Previously vs. newly infested df=1		Covariate df=1	
			F stat	P > F	F stat	P > F	F stat	P > F
A	Progrediens survival	Progredien crawler density	2.44	0.126	2.49	0.122	15.81	< 0.001
B	Settled Progrediens survival	Settled crawler density	1.34	0.255	0.19	0.668	12.05	0.001
C	Proportion not dispersing	Progredien crawler density	0.67	0.417	3.88	0.056	16.22	< 0.001
D	Progrediens fecundity	Progredien crawler density	0.36	0.552	8.09	0.007	15.23	< 0.001
E	Progrediens survival	Proportion of new growth	2.47	0.123	0.03	0.856	0.01	0.933
F	Total new growth (cm)	Progredien crawler density	102.15	< 0.001	15.72	< 0.001	0.18	0.673

¹Analysis of covariance on data presented in Fig. 1.4 (Proc Mixed, SAS 9.3). All densities and proportions surviving transformed to log₁₀. There were no significant interactions, so the terms were dropped from the models.

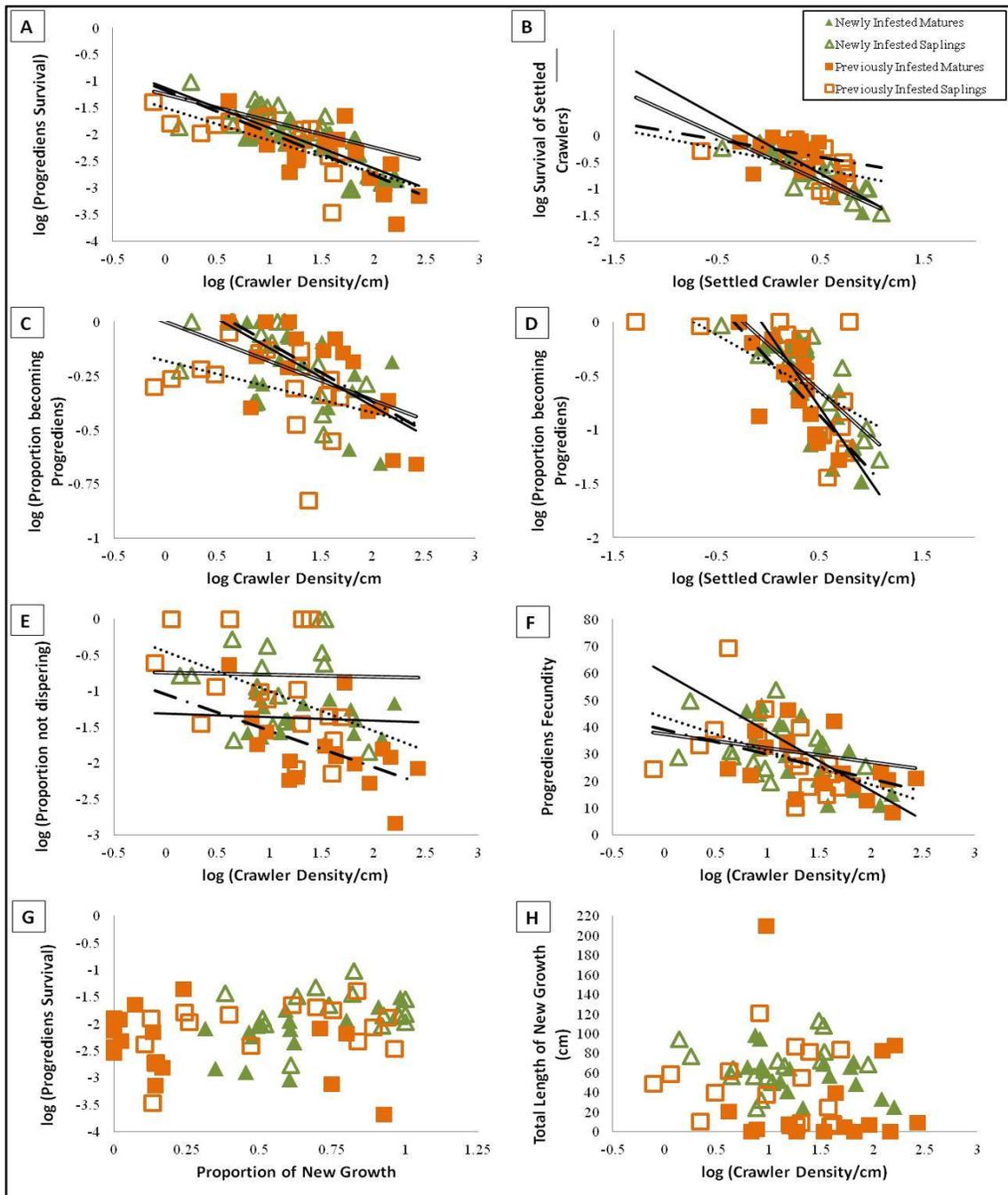


Figure 1.5. HWA density, fecundity, survival, and new hemlock growth during the spring generation in 2012. (A) Total density-dependent survival in the springtime progrediens generation; (B) Density-dependent survival of settled crawlers (C) Density-dependent survival from the production of sexuparae; (D) Density-dependent survival from sexuparae production following settlement of crawlers (E) Density-dependent dispersal survival; (F) Density-dependent fecundity decline in the progrediens generation; (G) Proportion of new growth on total progrediens survival; (H) Crawler density on amount of new growth (cm). Regression lines only plotted for significant covariate effects; — Newly Infested Mature, — Newly Infested Saplings, — · Previously Infested Matures, ····· Previously Infested Sapling Hemlocks.

Table 1.2. Statistical analyses¹ of HWA survival in the springtime progrediens generation from experiments performed in Massachusetts in 2012.

Fig 1.5	Dependent variable	Covariate	Sapling vs. Mature tree df=1		Previously vs. newly infested df =1		Covariate df=1	
			F stat	P > F	F stat	P > F	F stat	P > F
A	Progrediens survival	Crawler density	1.81	0.186	7.38	0.009	50.20	<0.001
B ₂	Settled progrediens survival	Settled crawler density	7.19	0.011	0.05	0.829	37.37	<0.001
C	Proportion becoming progrediens	Crawler density	3.83	0.057	1.57	0.216	28.44	<0.001
D	Proportion becoming progrediens	Settled crawler density	1.33	0.225	3.11	0.085	80.43	<0.001
E	Proportion not dispersing	Crawler density	16.37	<0.001	11.31	0.002	2.35	0.133
F ₃	Progrediens fecundity	Settled crawler density	0.32	0.574	0.02	0.876	29.24	<0.001
G ₄	Progrediens survival	Proportion of new growth	1.85	0.181	0.01	0.924	1.16	0.288
H	Total length of new growth (cm)	Crawler density	1.49	0.299	8.18	0.006	0.18	0.674

¹Analysis of covariance on data presented in Fig. 1.5 (Proc Mixed, SAS 9.3). All densities and proportions surviving transformed to \log_{10} . There were no significant interactions except as specified below, so the interaction terms were dropped from the models.

²Includes significant interactions: settled crawler density*previous infestation (F = 7.15, P = 0.012); statistics given are from a model that includes this interaction term.

³Includes significant interactions: previous infestation*tree age (F = 6.70, P = 0.013); statistics given are from a model that includes this interaction term.

⁴Includes significant interactions: previous infestation*tree age (F = 4.44, P = 0.041); proportion new growth*previous infestation*tree age (F = 3.86, P = 0.016); statistics given are from a model that includes this interaction term.

In both years of the sistens generation, the proportion surviving from those that did not disperse was density-dependent (Figs. 1.6A and 1.7A). In 2011 and 2012 aestivation survival was not related to the proportion of new growth produced on each experimental tree (Fig. 1.6B and 1.7B). Density-dependence was not detected during the

aestivation period in either year (Figs. 1.6 and 1.7C). We observed a density-dependent pre-winter survival of less than 10% on most experimental trees (\log survival < -1.0 , Figs. 1.6D and 1.7D). The difference between aestivation survival and pre-winter survival is that the latter also includes losses due to dispersal of sistens crawlers. Overwintering mortality was also density-independent (Fig. 1.6E and 1.7E). The net reproductive rate (R_0) for the entire generation in both years was significantly negatively density-dependent, and affected by previous HWA infestation (Fig 1.6F and 1.7F). The average R_0 for 2011 and 2012 was 0.50 and 0.64, respectively. Values of $R_0 < 1.0$ or $\text{Log}_{10} R_0 < 0$ indicate populations that declined over the one year period. Corresponding statistics are shown in Tables 1.1-1.4.

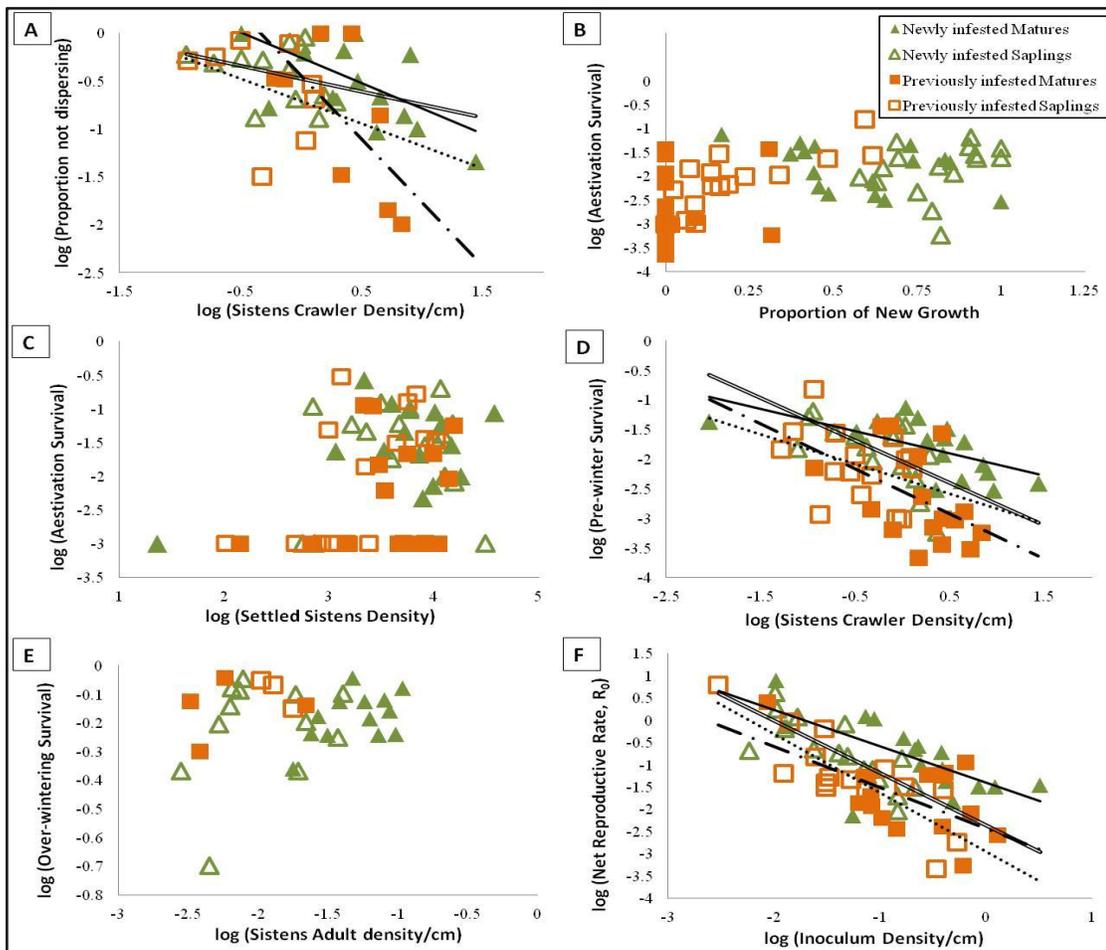


Figure 1.6. HWA density, survival, and new hemlock growth during the winter generation in 2011. (A) Density-dependent dispersal survival; (B) Proportion of new growth on sistentes aestivation survival; (C) Density of settled sistens crawlers on aestivation survival; (D) Density-dependent pre-winter survival; (E) Over-wintering survival; (F) Log net reproductive rate, $\log_{10}(R_0)$. Regression lines only plotted for significant covariate effects; — Newly Infested Mature, — Newly Infested Saplings, - - Previously Infested Matures, ····· Previously Infested Sapling Hemlocks.

Table 1.3. Statistical analyses¹ of HWA survival in the aestivating/over-wintering sistens generation from experiments performed in Massachusetts in 2011.

Fig 1.6	Dependent variable	Covariate	Sapling vs. Mature tree df=1		Previously vs. newly infested df=1		Covariate df=1	
			F stat	P > F	F stat	P > F	F stat	P > F
A	Proportion not dispersing	Sisten crawler density	0.30	0.590	3.45	0.075	5.28	0.030
B	Aestivation survival	Proportion of new growth	0.00	0.995	9.09	0.004	0.01	0.931
C	Aestivation survival	Settled crawler density	0.11	0.737	6.08	0.018	2.37	0.131
D	Pre-winter survival	Sisten crawler density	0.11	0.739	16.80	<0.001	12.66	<0.001
E	Over-wintering survival	Sisten crawler density	0.01	0.933	2.48	0.134	1.78	0.200
F	Net reproductive rate (R_0)	Inoculum density	1.44	0.237	17.06	<0.001	43.30	<0.001

¹Analysis of covariance on data presented in Fig. 1.6 (Proc Mixed, SAS 9.3). All densities and proportions surviving transformed to \log_{10} . There were no significant interactions, so the terms were dropped from the models.

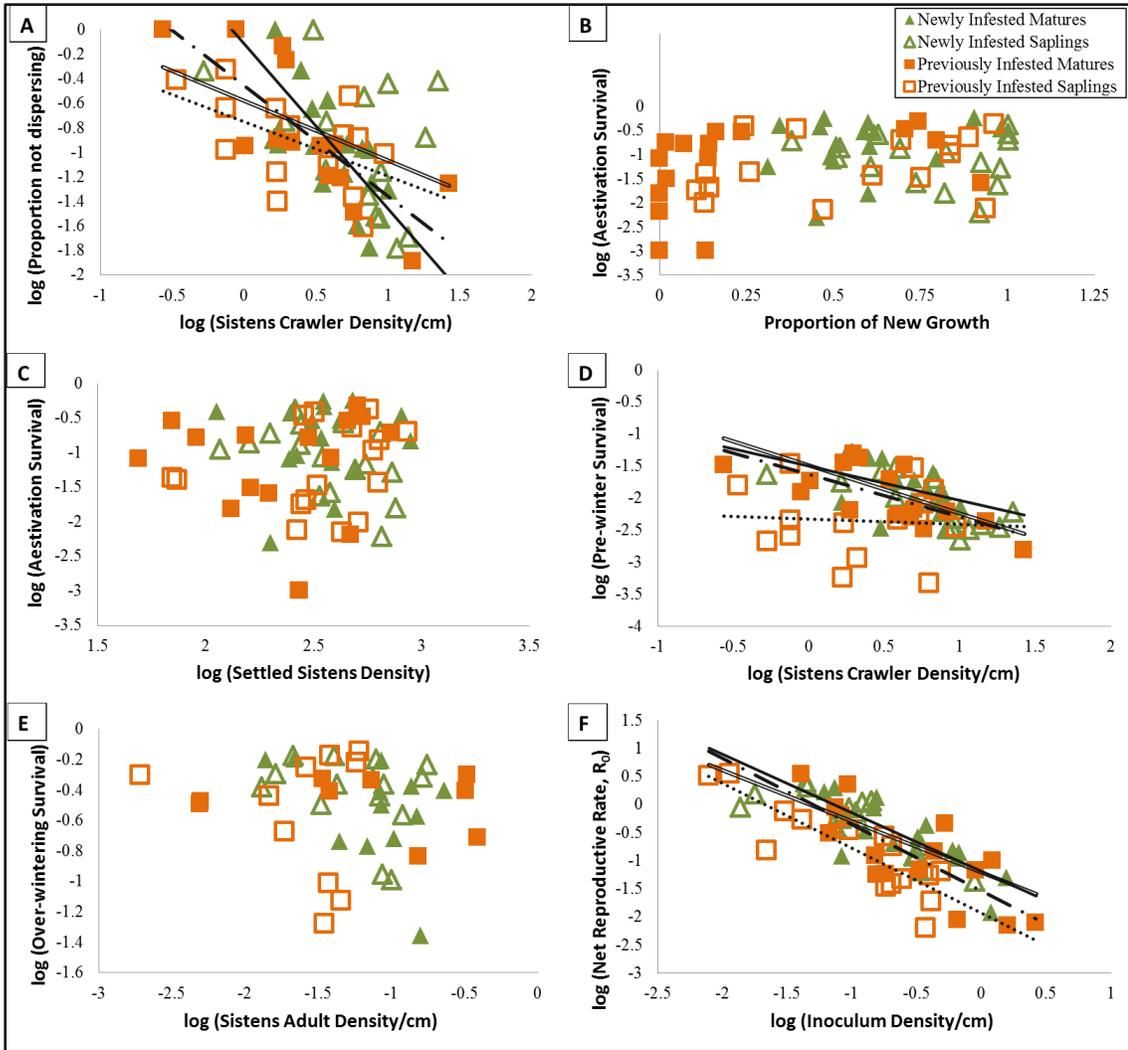


Figure 1.7. HWA density, survival, and new hemlock growth during the aestivating/overwintering sistens generation in 2012. (A) Density-dependent dispersal survival; (B) Proportion of new growth on sistentes aestivation survival; (C) Density of settled sistens crawlers on aestivation survival; (D) Density-dependent pre-winter survival; (E) Over-wintering Survival; (F) Log net reproductive rate, $\log_{10}(R_0)$. Regression lines only plotted for significant covariate effects; — Newly Infested Mature, — Newly Infested Saplings, — * Previously Infested Matures, — * Previously Infested Sapling Hemlocks.

Table 1.4. Statistical analyses¹ of HWA survival in the aestivating/over-wintering sistens generation from experiments performed in Massachusetts in 2012.

Fig 1.7	Dependent variable	Covariate	Sapling vs. Mature tree df=1		Previously vs. newly infested df=1		Covariate df=1	
			F stat	P > F	F stat	P > F	F stat	P > F
A ₂	Proportion not dispersing	Sisten crawler density	3.90	0.055	0.64	0.427	39.90	<0.001
B	Aestivation survival	Proportion of new growth	5.24	0.027	0.09	0.762	2.37	0.131
C	Aestivation survival	Settled crawler density	3.36	0.074	1.28	0.264	0.18	0.674
D	Pre-Winter survival	Sisten crawler density	3.46	0.070	4.72	0.035	13.36	<0.001
E	Over-wintering survival	Sisten adult density	0.02	0.882	0.51	0.479	0.24	0.629
F	Net reproductive rate (R_0)	Inoculum density	1.10	0.301	10.26	0.003	69.12	<0.001

¹Analysis of covariance on data presented in Fig. 1.7 (Proc Mixed, SAS institute 9.3). All densities and proportions surviving transformed to \log_{10} . There were no significant interactions except as specified below, so the interaction terms were dropped from the models.

² Includes significant interactions: sisten crawler density*tree age (F=7.02, P=0.011); statistics given are from a model that includes this interaction term.

1.4 Discussion

We manipulated HWA densities on 64 hemlock trees over two consecutive years, and demonstrated that overall HWA survival consistently declines with density in both the springtime and winter generations of HWA. In combination with previous work on HWA population dynamics (Paradis 2011), these results provide substantial evidence that density-dependent processes can regulate densities at levels below those which leads to hemlock death, at least in the northeastern United States. In contrast, overwintering mortality was not density-dependent in our study or in those of Trotter and Shield (2009) or Paradis (2011). As shown by the HWA population simulations by Elkinton *et al.* (2012), density-independent mortality in the overwintering generation, whether due to winter kill or egg predation, is immediately followed by pronounced density-dependent

mortality in the progredien stage that almost completely compensates for the preceding mortality in the winter or early spring. Thus, it is mortality in the springtime progrediens generation that accounts for most of the variation in observed densities of HWA adults in either the sistens or progrediens generation. These findings contradict the widely held belief that overwintering mortality accounts for the observed differences in HWA density in the northern versus southern regions. Our experimental manipulation of HWA population density in combination with our treatments of previously versus newly infested hemlocks and mature versus sapling trees have given further insight into the principal causes of this observed density-dependence.

Our results suggest that the proportion of progredientes that will become sexuparae is determined by current year HWA density, i.e., it is the density of the progrediens generation, rather than the density of the parental sistens generation that causes variation in the proportion of sexuparae produced. The latter effect was standardized across all treatments and densities by our inoculum procedure at the beginning of our experiment. This suggests that it is during the immature progredien stage that the switch to become a sexuparae is made. However, the fact that we observed no sexuparae production in 2011 but abundant production in 2012, suggests that sexuparae production may be at least partly determined by abiotic conditions. It is possible that environmental conditions acting on the parental sistentes provides the initial trigger, but that crawler density of the spring generation could be the secondary and final determinant of sexuparae production. Similarly, Moran et al. (2008) reported that both abiotic conditions as well as density were important triggers of sexuparae production in the sugarbeet root aphid (*Pemphigus populivenerae* Fitch).

Our results are consistent with those of McClure (1991), who demonstrated that HWA infestation causes a major reduction in new growth on hemlocks in the following year and that HWA survival is thereby reduced. He reported that sistens crawlers, which normally settle on new growth, were forced to feed on old growth where their survival was reduced. Like McClure (1991), we also found major reductions in new growth on previously infested trees, and a weak relationship between sistens aestivation survival and the proportion of buds with new growth in 2012. But we also found a more general effect of reduced survival in both the sisten and progrediens stages as well as lower progrediens fecundity on previously infested trees, at least in 2011. We suspect that other factors associated with previous infestation produced the reduced survival and fecundity. These could include a reduction in food reserves or production of chemical defenses that may be unrelated to, or poorly correlated with production of new growth. Various investigators have recently elucidated the defensive response of hemlocks to HWA infestation (Pezet et al. 2013).

In contrast, with previous infestation there was no consistent difference in HWA survival or performance on saplings versus branches of mature trees. This finding supports the idea that all of the important interactions between HWA and its hemlock host happen at the scale of the branch. In nature, we often observe both high and low densities of HWA on different branches of the same tree.

Paradis (2011 and unpublished data) reported densities from natural infestations that ranged from 72 - 388 progrediens crawlers/cm (mean = 167). In our experiment, densities ranged from 0.32 - 334.58 crawlers/cm (mean = 33.81), approaching densities of natural infestations only at our highest level of inoculum. It is possible that progrediens dispersal

was higher in our experiment than in natural infestations due to the inoculation procedure. Nevertheless, we observed density-dependent processes extending across two subsequent generations of HWA that resulted in an overall decline in population density on most trees as indicated by a \log_{10} (net reproductive rate) below zero (Figs 1.6F and 1.7F). We conclude from our results that these processes stabilize populations of HWA at densities well below the maximum densities recorded by McClure (1991) and Paradis (2011) in their studies of naturally occurring populations of HWA in the northeastern United States. We believe this phenomenon is the likely cause of the observed apparent stabilization of HWA populations in this region.

CHAPTER 2

SURVIVAL AND NEAR EXTINCTION OF HEMLOCK WOOLLY ADELGID IN A HEMLOCK PLANTATION

2.1 Introduction

In Chapter 1, data on the survival and fecundity of the hemlock woolly adelgid, *Adelges tsugae* Annand (hereafter HWA) is presented from experimentally-created HWA populations in a forest setting. Those results demonstrated density-dependent processes that stabilized the population densities and suggest that these processes may explain the long-term survival of HWA infested hemlocks, which has been widely observed in forests in the northeastern United States. These findings were consistent with those of McClure (1991) and Paradis (2011), who documented similar density-dependent mortality in naturally occurring populations of HWA in forests in southern New England.

These density-dependent processes are most pronounced in the springtime progrediens generations of HWA, but in the results presented in Chapter 1 such processes also seem to act within the overwintering sistens generation. These data suggest that any amount of mortality occurring in the overwintering sistens, whether caused by cold winter temperatures or by natural enemies, could be compensated for by the density-dependent processes occurring in the subsequent springtime progrediens generation (Elkinton et al. 2012).

Here, we present the results of an HWA inoculation experiment similar to that reported in Chapter 1, but in a 5-year old hemlock plantation, in contrast to the forest setting in Chapter 1. As in that study, adelgid-free hemlocks were inoculated with ovisacs of the overwintering sistens generation, and the survival and fecundity of two subsequent generations was recorded.

2.2 Methods

2.2.1 The experimental site

The experiment was conducted in a small hemlock plantation located at the University of Massachusetts Crops Research and Education Farm in South Deerfield, Massachusetts. The plantation was established in fall of 2007 with 1 meter tall hemlock seedlings from Cheshire Nursery Garden Center (Wallingford CT). The plantation comprised approximately 130 hemlocks arranged in a grid at 2 m spacing between trees and exposed to full sunlight on agriculturally modified soils. For the next four winters, trees were protected from winter wind damage with a 1.5 m tall barrier of landscape fabric. When the experiment was launched in May 2012, all of the hemlocks in this plantation were uninfested with HWA.

2.2.2 Experimental design

Trees were inoculated in the plantation by attaching 30 cm cut branches of eastern hemlock heavily infested with full ovisacs of the overwintering sistens generation to experimental hemlocks just before progrediens crawlers began to hatch. Infested inoculum branches of eastern hemlock about 30 cm in length were collected from a nearby forest on 17 April 2012, grouped into bundles of 1, 3, 10, or 30, placed in water-saturated foam overnight, and attached to experimental hemlocks the following day.

Experimental trees consisted of 16 uninfested hemlocks growing in a 4x4 block in the southeast corner of the plantation. All infested trees were of the same age and infestation history and were inoculated with a total of 30 twigs, with 1, 3, 10, or all 30 of them being heavily infested with healthy HWA ovisacs, and the remaining twigs free of HWA. The total number of sistentes on these inoculation twigs was determined by

counting them later thus converting the four density categories (1, 3, 10, 30 twigs) to a continuous variable.

2.2.3 Sampling method

Sampling methods were nearly identical to those employed in Chapter 1. Refer to Chapter 1, section 1.2.1 for HWA life history

After successful establishment of the progrediens crawlers, on 18 May 2012 the twigs used as the source of HWA inoculum were removed and brought to the laboratory to measure number and fecundity of the sistens generation on these twigs. Fecundity of the sistens adults was determined by the average number of chorions from hatched eggs in three woolly egg masses on each bundle of inoculum twigs. These numbers were then averaged across all trees in the stand. The number of progrediens crawlers produced on each experimental branch or tree was estimated by multiplying the number of previous generation sistentes on the inoculum branches by their mean fecundity.

To estimate sexuparae production in the spring generation, on 24 May 2012 sample twigs were cut from each inoculated tree and placed in water picks shortly following the establishment of spring generation crawlers. Populations of HWA on these cut twigs were reared in the laboratory in small Berlese funnels (BioQuip Products, Rancho Dominguez, and CA) to collect emerging sexuparae. Settled nymphs were counted under the microscope and the survival and fecundity of those adelgid that lived long enough to secrete wool and reproduce was determined. The number of progredientes that reached maturity was also recorded on each experimental hemlock in the field.

In early August, a branch containing visible settled sistentes from each experimental tree was selected and the terminal 30 cm were marked off. On each branch

terminal, the proportion of twigs with new growth, the total length of new growth (cm), and the number of settled sistentes were recorded. Aestivation survival was determined by recording the number of sistentes that survived to produce wool in December on both the 30 cm sample branches and on the entire experimental tree.

For each experimental tree at the plantation, the length and width of each limb at its widest point was measured, in combination with the total branch length of an individual branch from eight different trees. Plotting these two measures against each other yielded the following equation: $y = 0.281x + 601.1$, ($R^2=0.963$, Figure 2.8), which we used to obtain an estimate of total twig length on the entire tree from length x width measurements of all branches of the 16 experimental trees.

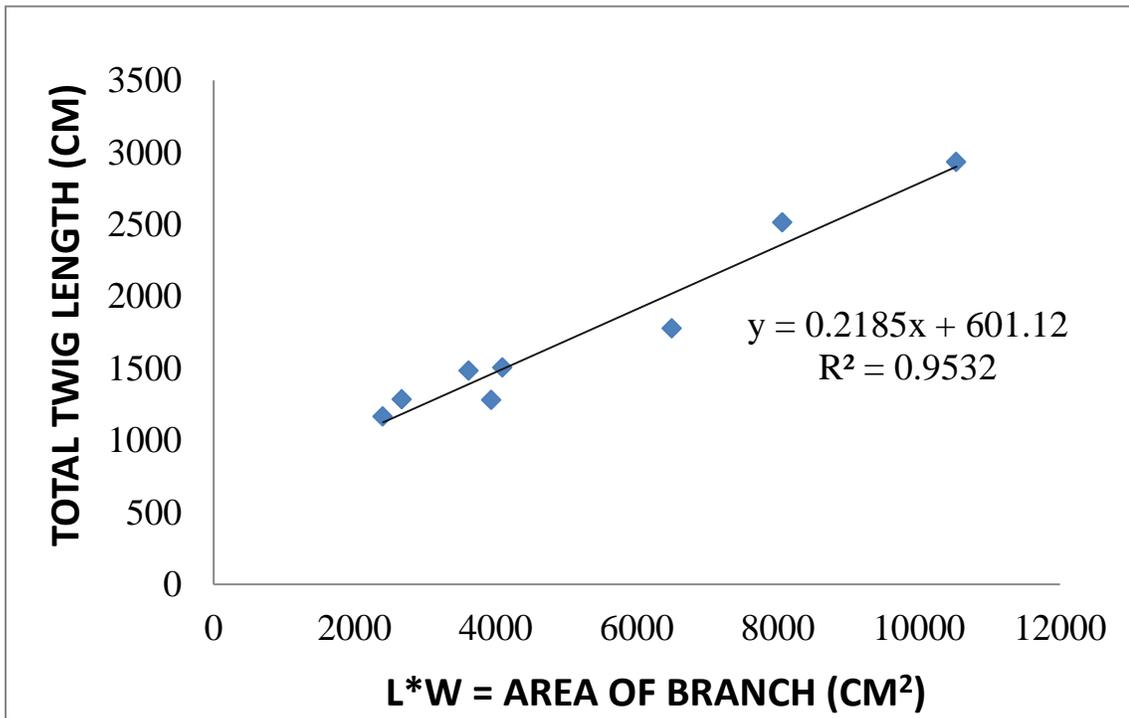


Figure 2.1. Linear regression of branch length x width measurements and total twig length sums per branch on each experimental hemlock.

In early July, iButtons (Maxim Integrated, San Jose, CA) were deployed at the site to collect air temperature data every 2 hours. Two iButtons were placed at the hemlock plantation in South Deerfield and four more were placed in the forest site at the Quabbin Reservoir to compare the climatic environments between the two sites. For June, the daily high and average temperatures were obtained from the South Deerfield and Pelham weather station (Plain Road East, KMASOUTH151; West Pelham Road, KMASHUTE2).

2.2.4 Data Analysis

The effects of HWA crawler and settled density as well as the amount of new growth on HWA survival and fecundity in the progredientes life stage were described using linear regression (Proc REG, SAS Institute 9.2). All densities and survival values were \log_{10} transformed to minimize inequality of variance.

2.3 Results

The survival of the springtime progrediens generation on the entire tree was not density-dependent (Fig 2.2A). However, survival of newly settled progredien crawlers was strongly density-dependent (Fig 2.2B), and the proportion becoming progredientes instead of sexuparae on the samples strongly decreased with increasing density (Fig 2.2C). The proportion that had become progredientes instead of sexuparae also declined with density on the sample branch collected from the field after sexuparae production was complete (Fig 2.2D). There was no significant impact of settled crawler density on adult progrediens fecundity (Fig 2.2E). The proportion of new growth on hemlocks was unrelated to the survival of the progrediens generation (Fig 2.2F), while progrediens

crawler density also had no effect on the amount of new growth produced by infested hemlocks (Fig 2.2G). Corresponding statistics are in Table 2.1.

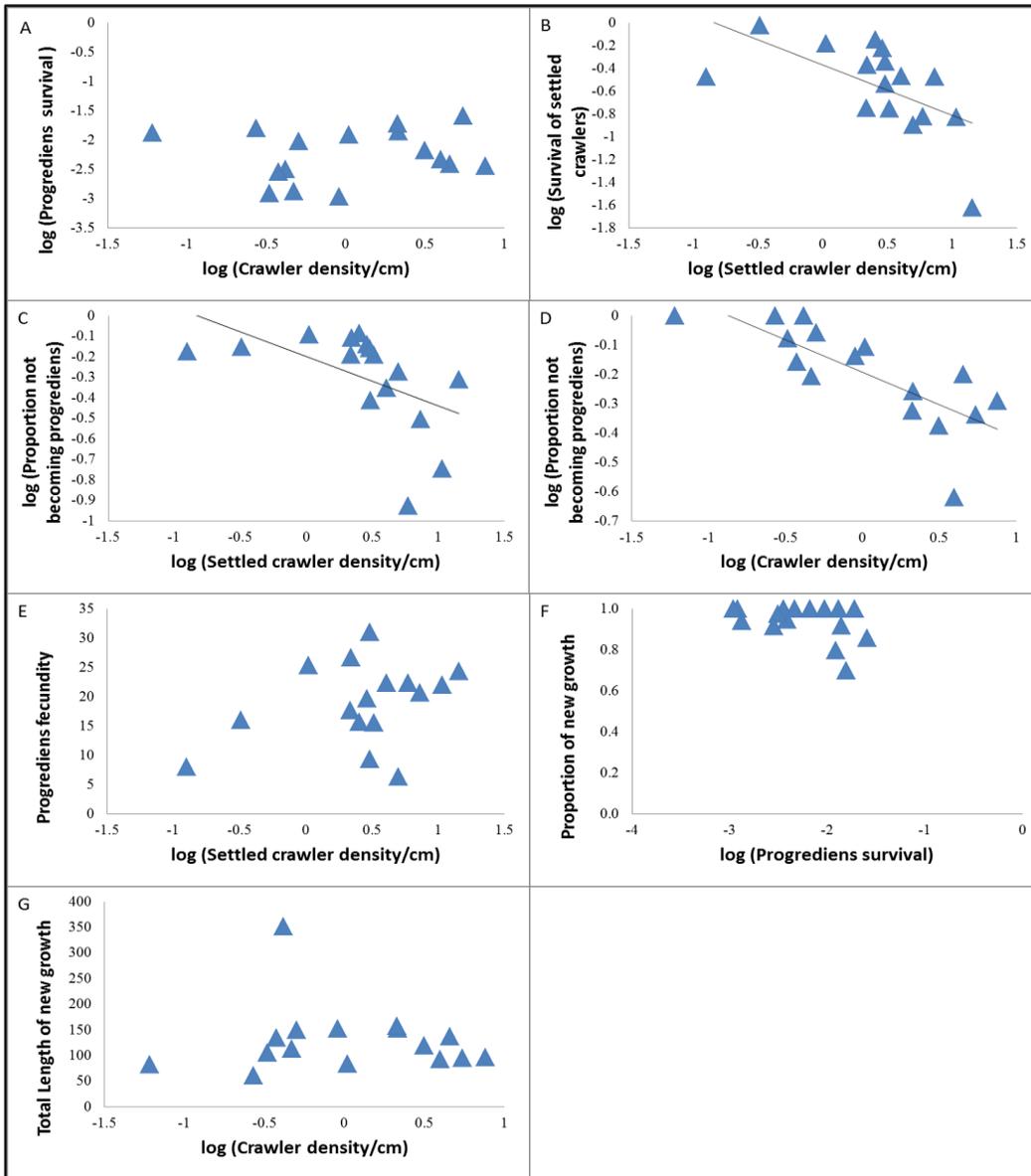


Figure 2.2. HWA density, fecundity, survival, and new hemlock growth from the hemlock plantation. (A) Total density-dependent survival in the progrediens generation; (B) Density-dependent survival of settled progredien crawlers; (C) Density-dependent survival from sexuparae production in funnels; (D) Density-dependent survival from sexuparae production from the field; (E) Density-dependent progrediens fecundity; (F) Effects of proportion of hemlock buds with new growth on progredien survival; (G) Effect of progredien crawler density on mean length of new hemlock growth (cm).

Table 2.1. Statistical analyses¹ on HWA survival in the springtime progrediens generation from an experiment performed at a hemlock plantation in 2012 from figure 2.2.

Fig 2.2	Dependent variable	Covariate	Intercept	Slope	R ²	Test of significance	
						F	P
A	Total Progrediens Survival	Crawler Density	-2.258	0.023	<0.001	0.01	0.922
B	Settled Progrediens Survival	Settled Crawler Density	-0.373	-0.443	0.354	7.69	0.015
C	Proportion becoming progrediens	Settled Crawler Density	-0.200	-0.241	0.270	5.17	0.040
D	Proportion becoming progrediens	Crawler Density	-0.125	-0.172	0.287	5.64	0.032
E	Progrediens Fecundity	Settled Crawler Density	16.974	4.517	0.123	1.96	0.184
F	Total Progrediens Survival	Proportion of New Growth (cm)	-0.267	-2.111	0.180	3.06	0.102
G	Total Length of New Growth (cm)	Crawler Density	130.977	-3.327	<0.001	0.01	0.925

¹Linear regression on data presented in Fig. 2.2 (Proc GLM, SAS Institute 9.2). All densities and proportions surviving transformed to log₁₀.

In early August, the number of settled sistentes was recorded on a terminal 30 cm sample branch with an average of 126 sistentes per branch. By early December, none of the aestivating sistentes on our sample branches had produced wool, indicating that aestivation mortality was 100% across all treatments. All 16 trees were searched, and no HWA was found on 13 trees. On three trees, a small cluster of HWA was found on the shaded inner portions of foliage near the bole of the tree.

When compared to the total amount of foliage (cm) on hemlock saplings in the forested Quabbin site, the plantation hemlocks were on average 9.3 times larger (data obtained from tree measurement data using the linear regression presented in Figure 2.1).

The daily minimum and maximum temperatures recorded were calculated to generate the absolute maximum and average temperatures at the plantation from May

through September 2012. Average monthly maximum temperatures at the South Deerfield plantation and the Quabbin were 34.6 °C and 30.1°C respectively. On average, the overall daily temperature at South Deerfield was 2.9°C warmer than the Quabbin (20.7°C and 17.8°C respectively) (Table 2.2).

Table 2.2. Temperature¹ differences between experimental sites.

	Hemlock Plantation, S. Deerfield MA		Quabbin Forest, Pelham MA	
MONTH	ABSOLUTE MONTHLY MAXIMUM	MONTHLY AVERAGE TEMPERATURE	ABSOLUTE MONTHLY MAXIMUM	MONTHLY AVERAGE TEMPERATURE
MAY	33.33	17.10	31.66	15.45
JUNE	35.56	19.26	33.33	17.76
JULY	36.75	23.10	31.62	21.69
AUGUST	36.00	26.96	29.50	19.45
SEPTEMBER	31.25	16.83	24.38	14.45
AVERAGE	34.58	20.65	30.10	17.76

¹Data from weather stations, May-June (Plain Road East, KMASOUTH15, West Pelham Road, KMASHUTE2) and from temperature data recorded every 2 hours by iButtons deployed at each site from July-September.

2.4 Discussion

This study examined the survival of HWA in relation to their density with the same methods as described in Chapter 1, but in a hemlock plantation exposed to open conditions and full sunlight. In contrast to previous observations of HWA population dynamics in a forest setting (Chapter 1), progredien survival within our plantation environment was not density-dependent, yet populations went nearly extinct during the

late summer/early fall aestivation period. Crawlers of the overwintering sistens settle preferentially on new growth in late June and enter an aestivation period during late summer through early fall (McClure 1991, Young et al. 1995, Butin et al. 2007). A likely purpose of this dormancy period is avoidance of activity during the hottest summer months (Salom et al. 2001). Intact branch counts confirmed that several hundred sistentes (a mean of 141.56 per terminal 30 cm sample per tree) had settled successfully and entered aestivation, but whole tree estimates of settled sistentes were not taken during this period because they are extremely small and difficult to count in the field. Instead, settled sistentes on 30 cm sample branches were counted to estimate aestivation survival. In December, these branches were revisited to see how many adelgid had survived aestivation to resume development and begin producing wool. None of these adelgid had begun producing wool, thus 100% of the aestivating sistens on these branches had died. Furthermore, there was no detectable survival on 13 of our 16 trees as determined by whole tree counts of maturing HWA. At the forested site from Chapter 1, overall survival during this period was 16%. The lower summer temperatures in the forest setting (Table 2.2) may account for the higher survival of HWA in this stage.

Host tree defenses may play a role in the survival of HWA. Lagalante *et al.* (2006) measured the terpene content of hemlock foliage across the growing season and showed that it was highest and most variable in late summer. They speculated that the aestivation phase of HWA occurred as a means to avoid these late-summer concentrations of terpenes. In a companion study to this one, E. A. McKenzie (unpublished) has shown that the terpene concentration in twigs and needles of the plantation hemlocks at S. Deerfield was several-fold higher than in the trees at our forest

site. Hemlocks growing in full sunlight may be able to allocate more resources to terpene production. It is not known, however, if terpenes play a role in HWA survival. Pezet *et al.* (2013) found no evidence that HWA induces terpene production in infested hemlocks, but he did find evidence for the induction of other compounds such as methyl salicylate, which operates in some plant species as a host defense signaling compound (Pezet *et al.* 2013).

There was no significant relationship between HWA survival or HWA density in either the length of the current year's new growth or the proportion of buds that produced new growth. These results matched those from the forested site in Chapter 1. The results from that study showed that it is the previous year's infestation of HWA that influence the length and proportion of the current year's new growth, but this did not affect HWA survival. In this study, none of our experimental trees had been infested with HWA in the previous year.

Experimental hemlocks at both the plantation and forest site were inoculated with approximately the same number of sisten ovisacs on the same day and from the same source population. However, plantation hemlocks had approximately 9.3 times the amount of hemlock foliage and were approximately twice as high as the saplings at our forest site, so the overall HWA densities were lower. In Chapter 1, density-dependent competition for resources or some other facet of interaction with the host tree probably caused the overall density-dependent mortality during the progredien stage. That same density dependent survival is evident in our branch samples of settled progredientes in the plantation experiment (Fig 2.2B), as well as in the forest experiment in Chapter 1. Similarly sexuparae production on these branches (Fig 2.2C, D) was density-dependent in

both experiments. In the plantation experiment reported here however, branches to estimate sexuparae production, as well as those selected for aestivation survival, were chosen among those with observable progredientes and sistentes and thus did not represent a random sample among all branches in the tree, most of which contained no observable HWA. On the smaller trees in the forest experiment all branches contained observable progredientes and sistentes, and the sample was selected at random. The lower overall densities in the plantation setting could explain why the same density-dependent processes over the entire progredien stage that were documented at the forest site were not observed in this experiment.

The differences in tree size resulted in substantial differences in progredien crawler density which ranged from 0.32 to 334.58 crawlers/cm (mean = 33.81) on forested hemlocks but only 0.10 to 10.25 crawlers/cm (mean = 2.54) in plantation hemlocks. Paradis (2011 and unpublished data) reported densities of progredien crawlers that ranged from 72 - 388/cm (mean=167) in her study of natural infestations of HWA in six populations in southern New England over a four year period. These densities were approached in the forest experiment (Chapter 1) only at the highest levels of inoculation, but the densities in the plantation experiment were lower by an order of magnitude at least. The mean density hemlock needles/cm was 14.89 (± 0.86 SE), for an average of 2.21 progredien crawlers/needle on forest hemlocks, and 0.14 crawlers/needle on plantation hemlocks. There were on average 0.20 settled progredien crawlers/needle on the forest hemlock samples, and 0.28 settled crawlers/needle on the plantation hemlock samples. As explained above, the settled crawler densities in the plantation were from branches with higher settled crawler densities than were typical for the tree as a whole.

Since HWA crawlers settle and feed at the base of hemlock needles, it may well be competition for space at the needle bases that limits the number of settled crawlers and the resulting maximum density HWA adults. However, both experiments have shown that density-dependent processes can affect the survival of settled crawlers at densities well below one adelgid per needle. The much lower overall densities in the plantation setting probably explains why the same density-dependent processes over the entire progredien stage that had been documented at our forest site, or that Paradis (2011) reported from her study of natural HWA populations, were not observed in this experiment.

In a similar HWA inoculation experiment in a hemlock plantation in West Virginia, Tobin et al. (2013) found no evidence of density-dependent survival during the progredien stage. The size of the trees were comparable to those in this plantation study, but the number on inoculation ovisacs was much lower, and also the fecundity of HWA sistens on the inoculation branches they used was about 10-fold lower. They found that even when hemlocks were inoculated with only one ovisac/tree, progredientes sometimes established successfully, and the subsequent initiation of the next generation (sistens) occurred in 35% of their trees.

The near-extinction of HWA on plantation trees during the aestivation sisten stage in late summer, despite many surviving adelgids from the preceding progredien generation, may help explain why HWA has spread so slowly in our region. HWA invaded central Massachusetts in the early 1990s, yet many trees remain uninfested nearly 20 years later (Orwig et al. 2012). This fact has usually been attributed to the high overwintering mortality that typically occurs in our region. Here, late-summertime

survival (less than 1%) was far lower than the over-wintering survival of HWA in the northeastern United States as documented in Chapter 1 and by Paradis (2011). These results do not explain why the rate of HWA spread and hemlock mortality are so much higher in southern regions where late-summer temperatures are presumably much higher.

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