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The Effects of Shade, Fertilizer, and Pruning on Eastern Hemlock Trees and Hemlock Woolly Adelgid

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Abstract: Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, an invasive insect native to the Pacific Northwest and Asia, is responsible for widespread health decline and mortality of native hemlocks (*Tsuga* spp.) in the eastern United States. Shading and fertilizer has been found to affect the survival and health of both HWA and hemlocks. These abiotic factors have been studied separately but not in combination. In this three year study, eastern hemlock trees (1–2 m tall) were treated with pruning, fertilizer, and shade to determine their effects on hemlock tree health and HWA survival and density. Shade cloths were erected over individual trees, granulated fertilizer was applied, and trees were pruned annually. The total number of HWA were counted during the sistens and progrediens adult stages on the low, mid, and high branches on the north, east, south, and west sides of each tree for three years. Survival of aestivating sistens was recorded in artificially, naturally, and unshaded hemlocks. The mean of percent tips alive, branches alive, and foliage density was used to calculate a hemlock health index (scale of 0–100). Shade cloth reduced solar radiation to the trees to levels similar to a naturally-forested hemlock canopy, but did not alter temperature. Trees exposed to shade alone and shade plus fertilizer maintained the greatest HWA density. On unshaded trees, branches on the west side of the tree had lower HWA densities and branches high on the tree had the lowest HWA densities. Pruning plus fertilizer and shading plus fertilizer reduced tree health. Shaded trees had reduced branchlet new growth length. Survival of summer aestivating sistens was nearly twice the survival under artificially- and naturally-shaded trees compared to unshaded trees. There was an inverse density-dependent survival response for aestivating HWA under artificially-shaded and unshaded trees but not naturally-shaded trees. Unshaded hemlock trees had lower HWA densities due to increased mortality of summer aestivating sistens. Unshaded trees had better health and longer new growth branchlets due to increased exposure to solar radiation and lower HWA densities. Silvicultural thinning of hemlocks in forest stands could increase direct sunlight reaching the trees and help decrease HWA densities and improve hemlock health.

Keywords: *Adelges tsugae*; aestivation; density-dependent; fertilizer; hemlock woolly adelgid; prune; shade; solar radiation

1. Introduction

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), was first recorded in the eastern U.S. infesting eastern hemlock (*Tsuga canadensis* (L.) Carr.) in the early 1950s in Richmond, VA [1]. Havill et al. [2] determined that the introduction originated from Japan. Since its introduction, HWA has caused significant health decline and death of eastern hemlocks from Maine to

Georgia [3–6]. The loss of this keystone species has resulted in significant changes to the aquatic and terrestrial ecosystems that are dependent on their presence [7–10].

Management of HWA in eastern forests has primarily focused on chemical [11] and biological control [12,13], while host resistance [14,15] and gene conservation [16] has received some attention. Imidacloprid and dinotefuran are the most commonly used insecticides that provide relatively quick and effective control that can persist for up to 7 years [17]. Hemlocks in heavily-visited areas in private and public parks and conservation areas are often treated to preserve this aesthetically and ecologically valuable species [18]. Biological control of HWA has the potential to offer long-term permanent management. Two predatory beetle species have been released in the eastern U.S., from the Pacific Northwest, *Laricobius nigrinus* (Coleoptera: Derodontidae) [19], and *Scymnus coniferarum* (Coleoptera: Coccinellidae) [20] and two from Japan, *Sasajiscymnus* (=*Pseudoscymnus*) *tsugae* (Coleoptera: Coccinellidae) [21,22] and *L. osakensis* [23]. Several other Coccinellidae (Coleoptera) and Chamaemyiid (Diptera) predators and an insect-killing fungus are also under consideration for use to control HWA [12]. Integrating chemical and biological control has also been found to be effective and compatible [24,25].

The HWA has two generations per year, sistens and progrediens [26,27]. The sistens generation begins as eggs in late June in Virginia [28]. The eggs hatch into first instar 'crawlers' which settle at the base of hemlock needles and remain there for the remainder of their lives. The first instars immediately enter aestivation [29] and do not begin developing until mid-October. They develop through four instars and reach the adult stage in early February. These adults lay progrediens eggs from February through April. The progrediens eggs hatch, settle, and develop though four instars and become adults in June and begin laying sistens eggs.

In studies where the authors artificially infested hemlocks with progrediens eggs in the spring, successful infestation of the progrediens generation usually exceeded 95%. However, the subsequent sistens generation density in the fall was observed to be consistently very low, suggesting that mortality of the aestivating sistens generation was high during the summer. Powers et al. [30] reported high infestation rates when artificially infesting hemlocks with progrediens eggs but the following sistens generation had very low densities and this decline was attributed to high light levels. Other studies have shown high heat events can decrease survival of summer aestivating sistens [31,32]. Shade or lack of shade may have an impact on HWA densities, with reports of higher HWA densities in low light conditions by Mayfield and Jetton [33], Sussky and Elkinton [31], and Hickin and Preisser [34]. Mech [32] reported increased mortality of aestivating sistens with increases in temperature, particularly above 30 °C. Brantely et al. [35] found higher HWA densities with lower light levels but also lower long-term carbon balance, suggesting that seedling health improves as light levels increase.

Increased rates of fertilizer have also been found to increase HWA density [36]. However, Joseph et al. [37] found conflicting results of fertilization of four hemlock species with varying responses of oviposition rates and densities. Also, the authors observed that pruned hemlock hedges tend to maintain relatively high densities of HWA. No reports to date have studied the effects of fertilizer, shade, and pruning in combination on HWA infestation levels. This study attempts to do that.

The effects of fertilizer, light, and pruning singly and in combination were examined to gain further insight into how these abiotic factors effect hemlock health and HWA densities. This study was conducted over a three-year period where the density of the two annual generations of HWA were measured at three different tree height levels in all cardinal directions. Tree health and branchlet new growth length were assessed annually. Survival of the aestivating summer stage in artificial shade, natural shade, and full sun was investigated. These treatments will also provide some insight on the effect of these abiotic variables on HWA and hemlocks and potentially add new tools to help manage HWA.

A secondary objective was to explore methods to increase HWA densities. *Laricobius nigrinus* has been field collected from the greater Seattle, WA area and directly released in the eastern U.S. It has also been mass reared in several insectaries in the East [38]. An alternative to mass rearing and collecting

L. nigrinus in its native range is to establish a field nursery in the East and collect *L. nigrinus* from this nursery for redistribution [39]. An increase in HWA would increase the biological control agent densities in plantation settings and increase collection numbers for reestablishment to other locations.

2. Materials and Methods

2.1. Hemlock Plantation

Laricobius nigrinus (Coleoptera: Derodontidae), native to the Pacific Northwest, has been released throughout the eastern U.S. since 2003 [12,19]. A field nursery to propagate this species was established by planting 360 eastern hemlock saplings in 2001 on a 0.4 ha pasture at the Virginia Tech Kentland Farm (37.2075° N, −80.5895° W) 16 km west of Blacksburg, VA, USA [39,40]. The hemlocks were initially infested with HWA in 2002, and subsequent years. Adult *L. nigrinus* were first released in the nursery in 2003 and are now established at this site at low densities due to consistently low HWA densities [39,40].

In October 2009, a second block (0.34 ha) of hemlocks (*T. canadensis*) was planted adjacent to the original planting. This planting had 10 blocks (12 × 20 m) with 30 hemlocks per block and 5 m between each tree. Trees were 1–2 m tall and uninfested with HWA when planted. The hemlocks in this second block were infested with HWA progrediens eggs in the spring of 2012. Hemlock woolly adelgids used for infesting the nursery were obtained from a heavily-infested hemlock hedge in Blacksburg, VA. When 5% of progrediens eggs began hatching (19 March 2012), two 30–40 cm long branches were cut and tied to hemlock branches to be used in this study with flagging on the north and south sides of each tree at mid height. A similar infestation technique was used by Butin et al. [41] and Powers et al. [30]. No deliberate introductions of *L. nigrinus* in this second hemlock block were made, since it was 10 m from the original planting and *L. nigrinus* adults should disperse into this planting naturally.

2.2. Experimental Design

Three treatments (shade, pruning, and fertilizing) were tested to determine the effect singly or in combination on HWA density and survival for a total of eight treatments: control (no shade, pruning, or fertilizer), pruning, fertilizer, shade, pruning plus fertilizer, shade plus pruning, shade plus fertilizer, and shade plus fertilizer plus pruning. Each treatment was replicated four times using four of the 10 hemlock blocks established in 2009. Within each of these four blocks, two randomly-selected trees received the same treatment, for a total of eight trees per treatment and 16 trees per block for a total of 64 trees in the entire study. Only trees with moderate to high HWA infestations were selected for treatment.

Shade was provided to randomly-selected individual trees with 3.7 × 3.7 m, 90% black woven shade cloth (Green-Tek Inc., Janesville, WI, USA) (Figure 1). In early May 2012, the shade cloth was erected directly over each tree using three, 3-m long, 1.3-cm diam. steel conduit. The four corners were tied with rope and attached to a steel rebar imbedded into the ground. The bottom edge of the shade cloth was approximately 1.0 m above the ground to allow access to the tree by *L. nigrinus* adults.

Results of the soil analysis recommended a mean of 20.2, 26.1, and 6.3 kg ha^{-1} of nitrogen, phosphorus, and potassium, respectively to be applied for optimal hemlock growth. Granulated fertilizer (Southern States, Richmond, VA, USA), 10% nitrogen, 10% phosphorus, and 10% potash, at a rate of 0.8 kg/tree, was applied on 13 March 2012, 20 June 2013, and 10 June 2014. This rate was four times the recommended rate based on soil sample analysis taken in each block, to ensure a response from the hemlock tree and possibly HWA. Soil samples were taken at the end of the study to determine the change in soil chemistry due to fertilization. Pruning was done on 20 May 2012, 24 June 2013, and 20 June 2014, when new foliage growth was 3–5 cm long, using a Stihl HS-56C hedge trimmer (STIHL, Virginia Beach, VA, USA) with a 0.6-m-long cutter head. Pruning was done after HWA assessments were made. The majority of the new growth was removed during pruning. Vegetation adjacent to the hemlocks was controlled by mowing and spraying with glyphosate.

Figure 1. Shaded and unshaded hemlock trees at the Virginia Tech Kentland Farm, Blacksburg, VA, USA.

2.3. Assessments

2.3.1. Solar Radiation and Temperature

To determine the amount of solar radiation blocked by the shade cloth, a solar radiation silicon pyranometer (Onset Corp., Bourne, MA, USA) was mounted under the shade cloth. The pyranometer measures the radiant intensity of light expressed as watts per square meter from 0 to 1280 W/m^2 over a spectral range of 300 to 1100 nm. A temperature sensor (Onset Corp., Bourne, MA, USA) was installed under one of the trees with a shade cloth. The temperature sensor was placed approximately 10 cm from the tip of a branch and under the branch where most HWA are found. A second pyranometer and temperature sensor were placed adjacent to this shaded tree with no shade cloth to record any differences in solar radiation and temperature between shaded and unshaded trees. This temperature sensor was placed inside a solar radiation shield to eliminate the effects of direct solar radiation on temperature. Data from these four sensors were recorded simultaneously every 30 min and stored in a HOBO (Onset Corp., Bourne, MA, USA) weather station.

To determine the degree of shading in a natural hemlock stand and the ambient temperature, solar radiation and temperature in a hemlock forest were recorded and compared to the solar radiation and temperature inside a solar radiation shield at the Kentland Farm weather station, 1.8 km west of the hemlock forest site. The solar radiation silicon pyranometer and temperature sensors were mounted in a hemlock stand under the branches of one hemlock tree (36 cm dbh) at 1.2 m from the ground from 1 June to 15 February 2017. Several other hemlocks of similar size were adjacent to the tree with the sensors. Hemlock woolly adelgid has been present at this site for 10 to 15 years. The hemlocks at this time were moderately infested with HWA. The trees were in moderate health with foliage densities 40–60% of an uninfested tree. It was not possible to measure solar radiation and temperature simultaneously in the forest and in full sun using the same data logger due to excessive distance from within the forest to an unobstructed location.

2.3.2. Adelgid Densities

To assess the effect of these treatments, HWA was sampled from 12 randomly-selected branches at three different heights (low, mid, and high) on the north, east, south, and west sides of each tree. The total number of HWA on the terminal 30 cm of each branch was recorded during the sistens (29 January 2013, 20 January 2014, and 9 March 2015) and progrediens (6 June 2013, 10 June 2014, and 18 June 2015) adult stages.

2.3.3. Tree Health Index and New Growth Length

The percent branch tips alive, branches alive, and foliage density was recorded in June 2014 and 2015. All of the parameters were rated on a scale of 0–100% in 5% increments so that a high value would indicate a healthy condition while a low value would indicate an unhealthy condition. The mean of these three measures (percent tips alive, branches alive, and foliage density) was used to calculate a hemlock health index (0–100) [42]. Percent live branch tips was the estimate of live hemlock branch tips relative to the branch tips that have no needles. Percent live branches was determined by estimating the number of branches that have live needles relative to the number of needleless dead branches [43]. Percent foliage density was an estimate of the remaining live foliage on the tree versus the foliage that would have been on the tree before defoliation that may have occurred in the last several years. This is the inverse of foliage transparency as defined by Schomaker et al. [43]. The mean lengths of new growth branchlets were measured on each of the 12 branches that HWA counts were made on in June 2014 and 2015.

2.3.4. Aestivating Sistens Survival

To determine the survival of aestivating sistens under artificially-shaded trees and those exposed to full sun, HWA-infested branches were collected randomly from respective treatments, after aestivation ended in late October 2015.

The survival of aestivating sistens under forested, naturally-shaded trees compared to those from unshaded trees was also investigated. In late October 2016, HWA-infested branches were collected from the hemlock tree in the forest with the pyranometer and temperature sensors and several other adjacent tees of similar size growing within a hemlock stand. Infested branches were also collected at the same time from the hemlocks growing in the open planation where this study was conducted. HWA on new growth were inspected under a dissecting microscope. Sistens producing fresh flocculent wax in late October were considered alive and those with no new wax were recorded as dead.

2.4. Data Analysis

2.4.1. Temperature and Solar Radiation

The mean temperatures and solar radiation were compared to determine if there were any differences in temperature with or without shade using the Student's Standardized *t*-test [44].

2.4.2. Density of HWA

Density of HWA was analyzed in PROC GLIMMIX (SAS 2008) following a Tweedie distribution with parameter $p = 1.5$ [45,46] with a log link function. This was a split-plot design with repeated-measures where the whole plot was the tree, the subplots were branches, and each tree was sampled repeatedly over multiple sampling dates (new branches each sample period). A random effect was placed on tree and sampling date within tree. To determine the effect of shade, fertilizer, pruning, height, and cardinal direction on HWA density, three-way interactions between shade, fertilizer, and pruning, and between height, cardinal direction, and shade, were fit along with the other factorial terms (main effects for shade, fertilizer, and pruning, and their two-way interactions). Additional main effects for date and block, to control for variation, were also included. Type III tests of fixed effects were checked for significance at $\alpha = 0.05$. If significant, post hoc differences of least square means were computed and checked for significance. Post hoc comparison *p*-values were adjusted for multiple comparisons using Tukey's method.

2.4.3. Tree Health Index

Tree health index (mean of percent tips alive, branches alive, and foliage density) was analyzed in PROC GLIMMIX [44] following a beta distribution with logit link function. A repeated measures design was used, where each tree was sampled repeatedly over multiple sampling dates and a random effect was placed on tree. For fixed effects, three-way interaction between fertilizer, pruning, shading, other factorial terms, a linear slope for HWA density, and a main effect for date and block, were analyzed to control for some variation. Type III tests of fixed effects were checked for significance at $\alpha = 0.05$. If significant, post hoc differences of least square means were computed and checked for significance. Post hoc comparison *p*-values were adjusted for multiple comparisons using Tukey's method.

2.4.4. New Growth Branchlet Length

Branchlet length in cm was analyzed in PROC GLIMMIX [44] following a gamma distribution with log link function. With respect to the other models and testing details, it was treated the same as HWA density, except that HWA density was included as a fixed effect with a linear slope to determine the effect of HWA density on growth.

2.4.5. Aestivating Sistens

Differences in survival of aestivating sistens on artificially- and naturally-shaded and unshaded branchlets, independent of density, were tested using Barnard's Exact Test for 2 $\times$ 2 tables (PROC FREQ) [44]. The effects of artificial and natural shade or no shade, HWA density, and interaction effects of treatment (shade or no shade) and density on survival of aestivating sistens were analyzed using PROC GLIMMIX [44].

3. Results

3.1. Solar Radiation, Temperature, and Soil Fertility

The HOBO weather station data logger failed several times during the study, but over 6000 measurements were made to provide ample data to make comparisons between shaded and unshaded temperatures and solar radiation. The shade cloth did not alter the ambient temperature within the tree compared to the temperature recorded in a shielded temperature sensor in full sun. The mean temperature was 9.1 and 10.0 °C with shade and without shade, respectively ($df = 6309$; $F = 1.0$; $p = 0.11$). The shade cloth significantly reduced solar radiation by 90% from a daily mean of 176.4 W/m^2 without shade to 18.1 W/m^2 with shade ($df = 7852$; $F = 85.9$; $p < 0.001$).

The mean daily solar radiation recorded in a natural setting within a hemlock canopy from 1 June to 31 October 2016 was 9.0 W/m^2 and the unshaded full sun Kentland Farm weather station solar radiation for the same time period was 200.1 W/m^2. Therefore, branches and subsequently HWA received only 4.5% (95.5% shade) of the full solar radiation in the natural hemlock stand. The naturally shaded trees were shaded to a slightly greater degree than the 90% shade cloth that was used in this study. Using the 90% shade cloth provided a very similar solar radiation level that would occur in a natural setting. After leaves dropped from the deciduous trees the mean solar radiation from 1 November 2016 to 15 February 2107 within the hemlock canopy was 16.0 W/m^2, a 44% increase in solar radiation.

While there was only a 0.9 °C difference between the mean ambient weather station temperature (21.8 °C) and the mean temperature in the naturally-shaded hemlock forest (20.9 °C) from 1 June 2016 to 31 September 2016, the temperatures were significantly different ($df = 5587$; $t = 7.9$; $p < 0.001$). This difference was attributed to the difference in the minimum and maximum temperatures of the two locations. The minimum temperature in the naturally-shaded hemlock forest and the weather station temperature located in a field was 7.6 °C and 5.8 °C, respectively. The maximum temperature in the naturally-shaded hemlock forest and the weather station temperature was 30.6 °C and 34.5 °C, respectively. The effect of the tree canopy shading in the forest moderated temperatures, resulting in warmer minimum and cooler maximum temperatures compared to an open exposed habitat [47].

After three years of annual application of four times the recommended rates of fertilizer, concentrations of phosphorus, potassium, and calcium in the soil were five to ten times greater than the recommended concentrations. The recommended rates in ppm for Christmas tree crops which include hemlock are: phosphorus 15–150, potassium 15–125, and calcium 125–250 ppm [48]. In the four treatments that were fertilized, phosphorus was 15 to 44 (437–1301 ppm) times the concentration of the control (30 ppm). Potassium was three to 11 (324–1385 ppm) times the concentration of the control (128 ppm). Calcium was equal to and over twice the concentration (953–1900 ppm) of the control (817 ppm).

3.2. Adelgid Densities

During the three years of sampling the sistens generation, a total of 4533 and 1904 HWA were counted in all the treatments that were shaded and not shaded, respectively. A close observation of HWA was necessary to accurately count the HWA. *Laricobius nigrinus* adults would have been visible during the HWA counting, but none were seen during sampling. This indicates that *L. nigrinus*, while present in the plantation, were at very low densities and had little or no impact on HWA density either on the trees that were shaded or unshaded.

In the first year of the study (2013), HWA sistens density was relatively low, ranging from 0.2 to 5.0 HWA/branch (Figure 2). The progrediens population in year one (2013) for all treatments was much greater than the previous sistens generation and ranged from 2.9 to 83.1 HWA/branch (Figure 2). In year two (2014), HWA densities for both generations were much lower than the sistens and progrediens generation in year one and ranged from 0.6 to 10.6 sistens/branch and 0 to 10.2 progrediens/branch, respectively (Figure 2). This overall reduction in density is likely due to the severe cold that occurred in January 2014 with a minimum of −19 °C and 88% HWA mortality at this site [49–51]. In contrast, the minimum temperature at Kentland during the winter of 2012–2013 was −12 °C.

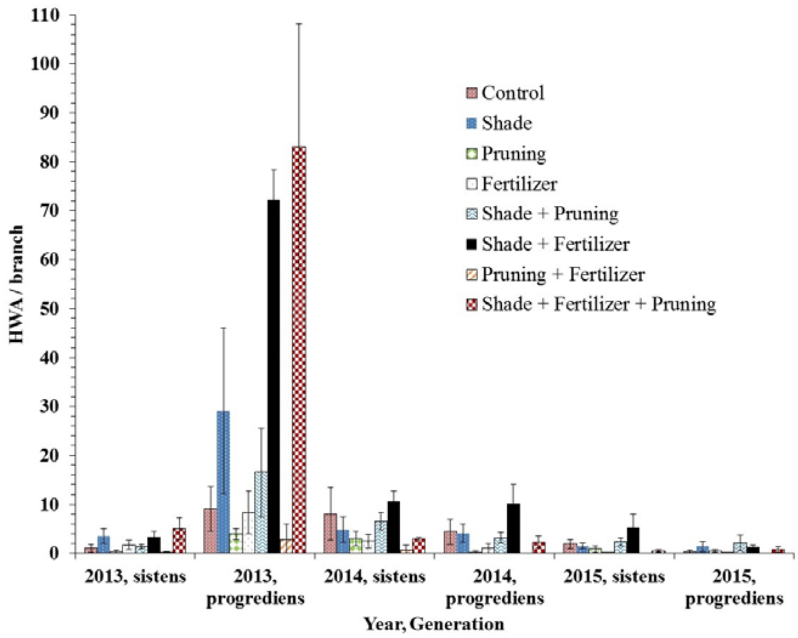


Figure 2. Mean density (±SE) of hemlock woolly adelgid (HWA) per hemlock branch with treatments of shade, fertilizer, and pruning.

Extremely low temperatures were experienced again in February 2015, with a minimum of −19 °C and 94% HWA mortality. This resulted in greatly-reduced HWA densities of 0 to 5.3 sistens/branch (Figure 2) in year three. Progrediens densities in year three were even lower than the sistens generation, with 0 to 2.1 progrediens/branch (Figure 2).

The higher order three-way interaction effects of shade plus pruning plus fertilizer did not converge in the initial analysis and was removed in the final analysis to allow for convergence of the model. When all sample dates (generations) were combined, the only significant interaction effects were prune * fertilizer (df = 1, 23; F = 6.2; p = 0.02) and shade * fertilizer (df = 1, 23; F = 6.2; p = 0.02) (Table 1). For the prune * fertilizer interaction the only significant difference found between post hoc comparisons of the least square means were unshaded trees that were pruned and fertilized (df = 23; T = −2.82; p = 0.04) (Table S1). These trees had lower HWA densities than trees that were unshaded and pruned but not fertilized. Pruning alone appeared to increase HWA densities while unshaded, pruned, and fertilized trees had lower HWA densities.

Table 1. Effects of shade, fertilizer, pruning, cardinal direction, and branch height location (low, middle, and high) on hemlock woolly adelgid density on hemlock, with all sampling dates combined.

Effect	Num. *df*, Den. *df*	*F*	*p*
Shade	1, 23	20.4	<0.001
Pruning	1, 23	1.6	0.22
Fertilizer	1, 23	2.7	0.12
Shade * Pruning	1, 23	0.3	0.57
Shade * Fertilizer	1, 23	6.2	0.02
Prune * Fertilizer	1, 23	6.2	0.02
Height	2, 1390	191.9	<0.001
Cardinal direction	3, 1390	27.6	<0.001
Shade * Cardinal direction	3, 1390	14.6	<0.001
Height * Cardinal direction	6, 1390	9.2	<0.001
Shade * Height	2, 1390	48.1	<0.001
Shade * Cardinal direction * Height	6, 1390	5.3	<0.001

For the shade * fertilizer interaction, two significant differences occurred between post hoc comparisons of the least square means. Shaded unfertilized trees had more HWA than unshaded fertilized trees (df = 23; T = 4.2; p = 0.002) (Table S2). Also, shaded fertilized trees had greater HWA densities than unshaded fertilized trees (df = 23; T = 4.8; p < 0.001). Therefore, fertilizer increased HWA density only when the tree was shaded and the effect of shading was greater than the effect of fertilizer. Pruning had no effect on HWA density of shaded trees. The effect of fertilizer on HWA density was dependent on shading and pruning. Shading had the greatest impact in increasing HWA densities.

The three-way interaction between cardinal direction, shading, and height had a significant impact on HWA density (df = 6, 1390; F = 5.3; p < 0.001) (Table 1). To determine the effects of natural shading caused by the tree itself, comparisons of the least square means of the unshaded trees found that the high height tended to have the lowest HWA density and the western side of the trees also had lower HWA densities (Figure 3, Table S3).

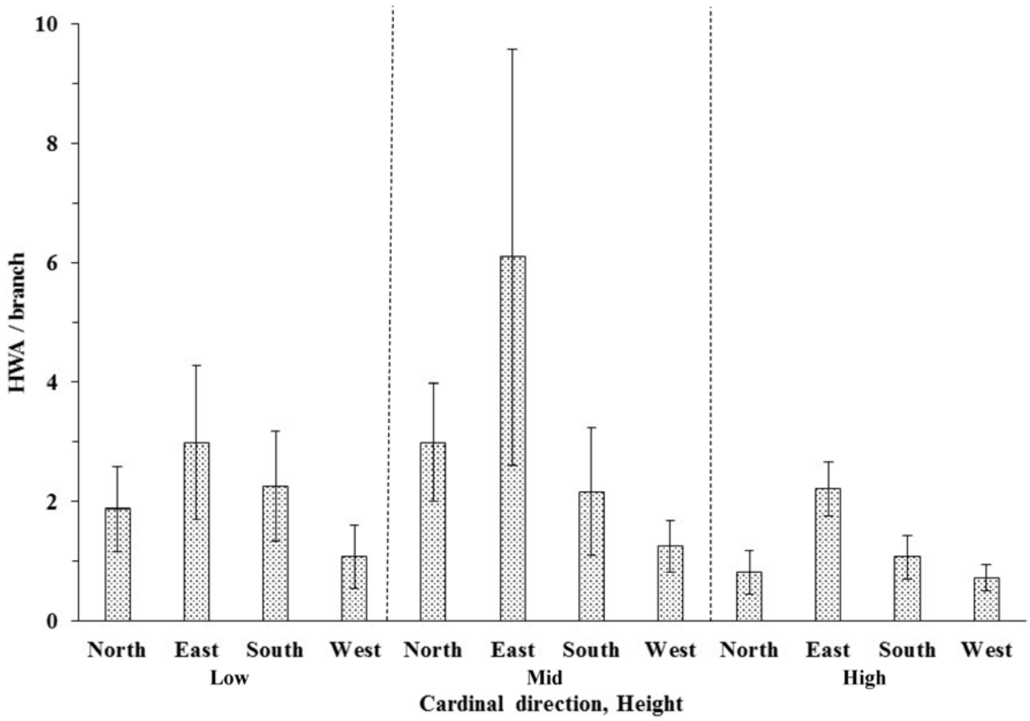


Figure 3. Mean (±SE) density of hemlock woolly adelgid (HWA) per branch on the four cardinal directions at three different heights on unshaded hemlocks, with all treatments combined.

3.3. Tree Health and New Growth Length

A significant interaction occurred in tree health in the shaded * fertilized trees ($df = 1, 23$; $F = 7.7$; $p = 0.01$) and prune * fertilized trees ($df = 1, 23$; $F = 5.8$; $p = 0.02$) (Table 2, Figure 4). Unshaded trees that were pruned and fertilized had significantly worse health than trees that were only pruned ($df = 23$; $T = -4.1$; $p = 0.003$), only fertilized ($df = 23$; $T = -2.8$; $p = 0.04$), or had no pruning or fertilizer ($df = 23$; $T = -3.3$; $p = 0.01$) (Table S4). The combination of pruning and fertilizer reduced tree health. Trees that were shaded and fertilized had worse health than trees that were shaded and not fertilized ($df = 23$; $T = -4.9$; $p < 0.0001$), fertilized and unshaded ($df = 23$; $T = -9.5$; $p < 0.0001$), and unshaded and unfertilized ($df = 23$; $T = -9.5$; $p = 0.001$) (Table S5). Fertilization of shaded trees appeared to be detrimental to tree health. Fertilization had no effect on unshaded trees ($df = 23$; $T = -0.31$; $p = 0.99$) (Table S5). These hemlock trees received high rates of fertilizer annually. Over-fertilization of shaded hemlocks may be too detrimental to hemlock health. Hemlock woolly adelgid did have an effect in reducing the tree health index ($df = 1, 89$; $F = 4.3$; $p = 0.04$) (Table 2).

Table 2. Effects of shade, fertilizer, pruning, and hemlock woolly adelgid on hemlock health index (mean of percent tips alive, foliage density, and live branches) and new branchlet growth length with all sampling dates combined.

Effect	**Health Index**			**New Growth Length (cm)**		
	Num. *df*, Den, *df*	***F***	***p***	**Num. *df*, Den, *df***	***F***	***p***
Shade	1, 23	101.1	<0.0001	1, 26	111.9	<0.0001
Pruning	1, 23	1.8	0.19	1, 26	1.6	0.21
Fertilizer	1, 23	10.7	0.01	1, 26	5.1	0.03
Shade * Pruning	1, 23	2.3	0.15	1, 26	4.2	0.05
Shade * Fertilizer	1, 23	7.7	0.01	1, 26	6.3	0.09
Prune * Fertilizer	1, 23	5.8	0.02	1, 26	1.3	0.26
Shade * Pruning * Fertilizer	1.23	0.13	0.72	1, 26	0.4	0.54
Hemlock woolly adelgid	1, 89	4.3	0.04	1, 1,397	10.7	0.001

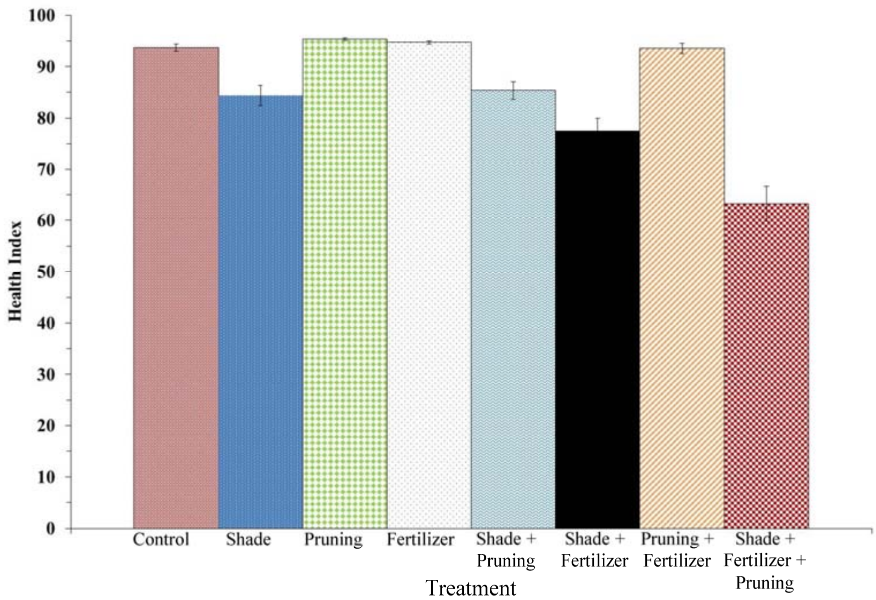


Figure 4. Mean (±SE) hemlock health index (mean of percent branches alive, foliage density, and tips alive), with both sampling years combined.

Branchlet new growth length was significantly different for trees that were shaded ($df = 1, 26$; $F = 119.1$; $p < 0.0001$) (Table 2, Figure 5). Least squares means indicated that shaded trees had significantly shorter new growth branchlets than unshaded trees ($df = 23$; $T = -10.6$; $p < 0.001$). Fertilizer-only trees were also significantly different ($df = 1, 26$; $F = 5.5$; $p = 0.03$). Least squares means indicated that fertilized trees had slightly shorter branchlet lengths than unfertilized trees ($df = 26$; $T = -2.3$; $p = 0.03$). Hemlock woolly adelgid had a significant impact in reducing new growth branchlet length ($df = 1, 1397$; $F = 10.7$; $p = 0.001$).

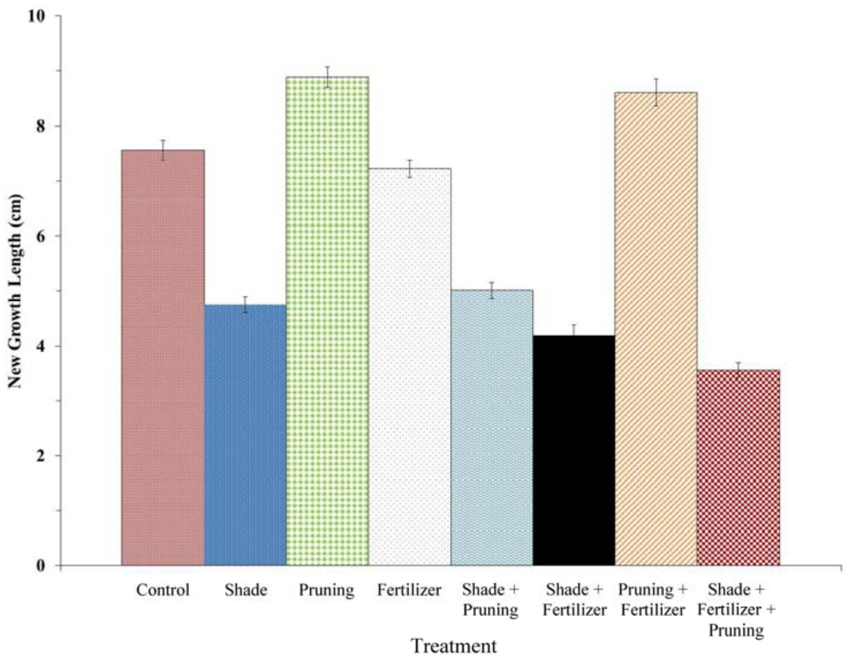


Figure 5. Mean (± SE) length (cm) of hemlock new branchlet growth with both sampling years combined.

3.4. Aestivating Sistens Survival

Significantly more aestivating sistens survived on artificially-shaded trees (41%) than unshaded trees (22%) (Z = −73.2; $p < 0.001$) (Table 3). Aestivating sistens survival was also greater (Z = −67.3; $p < 0.001$) on naturally-shaded trees (45%) compared to unshaded trees (25%). Survival of sistens under artificial and natural shade was nearly twice the survival rate without shade.

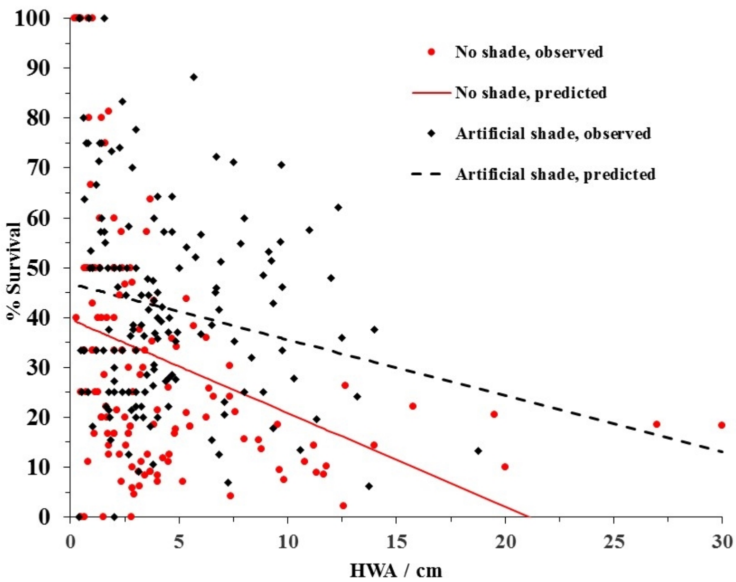
Table 3. Effect of artificial (shade cloth) and natural shade (forested setting) on the survival of aestivating hemlock woolly adelgid (HWA) sistens, independent of branchlet HWA density (Barnard's Exact Test).

Treatment	Total HWA	Dead [1]	Alive [2]	% Survival	*Z*	*p*
Artificial shade	2992	1766	1226	41.0	−73.2	<0.001
No shade	2371	1855	516	21.8		
Natural shade	1833	1012	821	44.8	−67.3	<0.001
No shade	2695	2027	668	24.8		

[1] The number of HWA that did not break aestivation. [2] The number of HWA that did break aestivation.

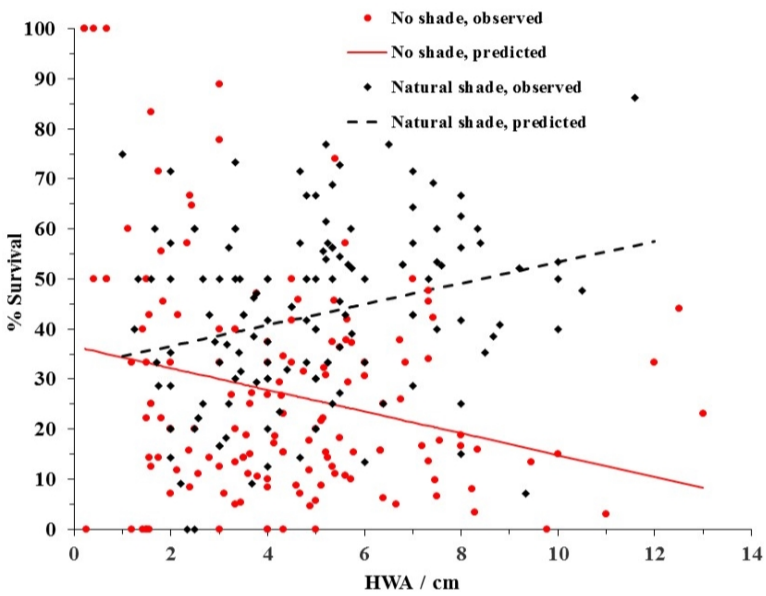
Analysis of survival based on density of HWA per cm in the artificial shade vs. no shade, showed that treatment (shade or lack of shade) (df = 1, 294; F = 73.6; $p < 0.001$) and HWA per cm (df = 1, 294; F = 53.1; $p < 0.001$) had a significant effect on survival rate (Table 4). Aestivating sistens had an inverse density-dependent response to survival both on artificial shade and no shade branches, with greater densities of aestivating sistens resulting in lower survival both on artificially-shaded and unshaded trees (Figure 6a). There was no treatment * density interaction effect (df = 1, 294; F = 0.1; p = 0.83), indicating that the survival rate of aestivating HWA was the same for both artificially-shaded and unshaded trees.

However, in contrast to the artificial shade, there was an interaction effect in the forested natural shade study with aestivating sistens surviving differently (df = 1, 286; F = 30.4; p = <0.001) on naturally-shaded branches compared with unshaded branches (Table 4). When aestivating sistens were not shaded, the density-dependent response was similar to the artificial shade and no shade analysis above, with survival decreasing as the HWA density increased (Figure 6b). However, under natural shade, aestivating sistens survival increased as HWA density increased.



(**a**)

Figure 6. *Cont.*



(**b**)

Figure 6. Observed and predicted density and survival of aestivating HWA sistens in the (**a**), artificial shade and no shade study and (**b**) natural shade and no shade study.

Table 4. Effects of treatment (shade or no shade) and density of hemlock woolly adelgid (HWA) per cm on new growth branchlets of aestivating HWA sistens.

Effect	Num. *df*, Den, *df*	*F*	*p*
Artificial Shade			
Treatment	1, 294	73.6	<0.001
HWA density	1, 294	53.1	<0.001
HWA density * Treatment	1, 294	0.1	0.83
Natural Shade			
Treatment	1, 286	0.1	0.72
HWA density	1, 286	0.8	0.05
HWA density * Treatment	1, 286	30.4	<0.001

4. Discussion

The shade only and shade plus fertilizer trees had significantly greater HWA densities than trees growing in full sun with no fertilizer or pruning. Shade enhanced the effect of fertilizer on HWA since there was no difference in HWA density in the unshaded fertilizer only treatment. Shaded trees declined in overall health index (percent branch tips alive, foliage density, and live branch) and branchlet length compared to the unshaded trees. The greatly-increased fertilizer concentrations in this study did not improve tree health of shaded trees. The added fertilizer did not offset the negative impacts of HWA feeding or the negative effects of shading to tree health. Over-fertilization may have been detrimental, as unshaded, pruned, and fertilized trees had reduced health and shorter branchlets. McClure [36] reported increasing HWA density with increased nitrogen, but nitrogen did not benefit the hemlock enough to offset the negative impacts of HWA feeding. McClure [36] also found increased survival of HWA nymphs, increased egg production, HWA density, and hemlock new tip growth with increased levels of nitrogen. Joseph et al. [37] found conflicting results of fertilization of four hemlock species with varying responses of oviposition rates and densities. Jones et al. [42] examined the effect of tree health on health and fecundity of HWA and found results somewhat in conflict of McClure [36] and Joseph et al. [37]. In the Jones et al. [42] study, moderately HWA-impacted trees had greater HWA fecundity but were less healthy (based on nutrient content) than lightly-impacted trees.

Pontius et al. [52] examined the foliar chemistry of hemlock needles and found that higher concentrations of N and K in the needles may enhance hemlock palatability, thus increasing HWA densities, while higher concentrations of Ca and P may reduce HWA density. Their study did not report soil concentrations of these elements, and our study did not analyze needle chemistry, however, a high level of these elements in the soil may indicate a similarly high level in the hemlock needles. Although the fertilizer applied to the soil could have taken other routes, such as loss to nitrification, leaching, or microbial activity in the soil, the soil ppm of N, K, Ca, and P were all well above the recommended ppm of these elements in the fertilized trees. Based on the results of Pontius et al. [52], the high levels of N and K, which were reported to benefit HWA, may have been counteracted by the negative effects of the high levels of Ca and P in this study. This may have resulted in only a minor increase of HWA density with fertilization of shaded trees.

The effect of fertilizer appears to have some impact on HWA density and tree health but its effect appears to vary, as other studies have shown. These studies were conducted at different locations, habitats, growing conditions, soil fertility, age, and previous HWA infestation rates and durations. The cyclical dynamics of the impact of HWA on tree health and the impact tree health has on HWA is a difficult problem to discern and more study is needed.

The unshaded trees were actively growing in the summer whereas the shaded trees were growing at a slower rate, as indicated by the shorter branchlet lengths of the shaded trees compared to the unshaded trees. This reduced new branchlet growth is supported by Brantley et al. [35] where they reported reduced photosynthetic efficiency and shorter branchlet length with reduced light levels. The effect of increased fertilizer in the current study was not as great as the effect of shade in increasing HWA density. Shade may have lowered the growth rate of hemlocks, reducing the amount of nutrients used by the tree and instead allowing more of the nutrients to accumulate in the stems and needles to be used by HWA. The survival of aestivating first instars in full sun may have been impacted by both the increased solar radiation and lack of access to nutrients in the actively-growing unshaded hemlocks.

Among the unshaded trees, the pruned and unfertilized trees had greater HWA densities than trees that were pruned and fertilized. Fertilizer reduced HWA densities in pruned unshaded trees. While the authors have observed high densities of HWA on pruned hedges, the timing of when pruning occurs likely impacts HWA density. Pruning in this study was done several weeks after the counts were made, so as not to remove any HWA and alter the counts of the adult HWA progrediens. The sistens generation settles on the new growth that was pruned in mid-June and would account for the differences in HWA sistens density in the pruned treatments. The HWA remaining after pruning were the aestivating sistens that had settled on the new growth that was shorter than the new growth that was pruned off and growing in the interior of the tree. Pruning and fertilizing tress growing in unshaded open habitats may be a tool in reducing HWA.

Trees growing in shade had greater HWA densities, shorter branchlets, and overall reduced health. The results of the interaction analysis on the effect of HWA on tree health showed that HWA did have an effect on reducing tree health and the combination of shade and high HWA densities both contributed to reduce tree health.

The height and cardinal direction effects of unshaded trees on HWA density were not as pronounced as the effect of the shade cloth. The hemlock branches near the top of the trees, regardless of cardinal direction, were exposed to the full solar radiation and had no shading from adjacent trees. These branches had the lowest HWA densities. The mid- to low-height branches likely received slightly less solar radiation due to shading from branches above. The mid and low branches had greater HWA densities than the highest branches receiving full sun. However, while the north side of the tree would be expected to have had the lowest solar radiation and consequently greater HWA densities, the west side of the tree had lower HWA densities than the north, south, and east sides. Multiple pyranometer measurements on all sides and heights of the tree would provide a better understanding of the actual amount of solar radiation received by the branches at the different cardinal directions and heights.

Sussky and Elkinton [31] reported findings similar to our study, with respect to greater HWA densities in shade, with densities of HWA in the forest (shade) ten times greater than densities in the planation (full sun). They found that aestivating sistens had greater survival in the forest (16%) compared with sistens on trees in a planation setting exposed to full sun that had no survival [31]. Mayfield and Jetton [33] and Hickin and Preisser [34] reported greater densities of newly settled sistens with lower light levels. The survival of aestivating sistens was not examined in these two studies. But the higher successful settlement rate of sistens in low light plus our findings of higher survival of aestivating sistens in low light, would add credence to there being overall greater densities of HWA in low light conditions. Jones et al. [42] reported survival of 40% of aestivating sistens in a forested setting. A very similar survival rate (41%) was found in our study.

Mech [32] reported a positive correlation between increasing temperatures, duration of exposures, and HWA mortality of aestivating sistens, with mortality markedly increasing above 30 °C. From June to October 2016, the daily maximum temperature without shade was at or above 30 °C, for 41 days. During the same time period the daily maximum temperatures recorded in the forest with natural shade was above 30 °C only nine times, due to the moderating effects of the forest canopy [47]. In the artificially-shaded trees, there were 38 days above 30 °C in 2015 when the aestivating sistens survival study was done.

To study the effect of temperature on survival of aestivating HWA sistens, Mech [32] conducted the experiment in growth chambers with artificial light and no solar radiation. While in our study there were no differences in temperature in the artificially-shaded trees and unshaded trees, there was a 90% difference in solar radiation and survival. Similarly, in the naturally-shaded trees there was a slightly lower temperature regime but a substantial difference in solar radiation (95%) and survival compared to unshaded trees. Perhaps at temperatures above 30 °C, temperature is more detrimental to survival and below 30 °C solar radiation has more of an impact. The combination of temperatures above 30 °C plus higher solar radiation of unshaded trees would have an even greater impact.

Sistens crawlers that hatch in June settle at the base of needles and enter aestivation from June to October [3,29,41,53]. During the summer, when solar radiation is greatest, HWA aestivates as first instars. The only wax on the summer aestivating first instars is along the lateral edge of their body. Without a wax cover, the insect may be exposed to more solar radiation that can impact its cuticle and raise its temperature and cause higher levels of mortality. Greater exposure to direct sunlight may be detrimental to an herbivore adapted to feed on a shade-tolerant species such as hemlock. Higher solar radiation levels could lead to increases in body temperature, exposure to ultraviolet light, or alteration of the host plant physiology or quality [54,55]. Hemlock woolly adelgid within the forest are shaded by the canopy of hemlocks and deciduous trees during the summer and this shading may protect the exposed aestivating sistens from solar radiation and higher temperatures. In the study, when the deciduous trees lost their leaves, solar radiation increased by 44% within the forest under the hemlock tree. Although the amount of increase in solar radiation after leaf senescence would vary considerably depending on the density of the hemlock canopy and surrounding deciduous trees, when leaf drop occurs, HWA begins producing wax which may protect it from the increased solar radiation. Jones et al. [56] found that the HWA wax contains compounds that are predator deterrents. Although temperatures are much reduced during the fall, winter, and early spring compared to the summer, the production of wax may also reduce the effects of solar radiation. More study on the interaction of temperature and solar radiation on the year-round survival of HWA would be warranted.

Survival of aestivating sistens was inversely density-dependent, with lower survival with higher densities of sistens in both the artificially-shaded trees and trees in full sun. However, there was a density * treatment (shade or no shade) effect in the natural shade study, indicating that the density-dependent survival rate was different under the natural shade compared to full sun. Survival of sistens decreased as density increased when there was no shade and survival increased as HWA density increased when under natural shade. The maximum density of aestivating sistens in the natural shade vs. unshaded trees was 50% less than the highest density in the artificial shade vs. full sun study (Figure 6a,b). These lower densities in the natural shade study may have diluted the density-dependent effect.

Based on the findings of this study, a silvicultural tool to reduce HWA density in the forest setting would be to expose hemlock trees to more sun by cutting adjacent trees to increase solar radiation exposure to the hemlocks. Brantley et al. [35] also made a similar recommendation based on their studies of the effect of light on HWA and hemlocks. This could increase the mortality of summer aestivating sistens by exposing them to more solar radiation and increased temperatures leading to reduced HWA densities. The degree of thinning to achieve lower HWA densities would require further study. In landscape plantings, pruning and fertilizer could also be used to reduce HWA in sunny locations. Pruning of new growth should be done after the sistens eggs have hatched and the sistens crawlers have settled, to maximize the removal of this generation. The settled sistens usually settle on the new growth by early July.

5. Conclusions

Trees exposed to shade alone and shade plus fertilizer maintained the greatest HWA density. Shading reduced tree health and length of new branchlet growth. Summer aestivating sistens survival under artificially- and naturally-shaded trees was nearly twice the survival under unshaded trees. Unshaded hemlock trees had lower HWA densities due to increased mortality of summer aestivating sistens, improved tree health, and increased growth of new branchlets. To decrease HWA densities, this study suggests exposing hemlock trees to more sun by cutting adjacent trees to increase solar radiation exposure. This would increase summer aestivating sistens mortality and increase photosynthetic activity in hemlocks. To increase HWA density, in a field nursery for HWA predator rearing, shading and to a lesser extent fertilizing hemlocks may be worthwhile management techniques. Using shade cloth may be impractical and costly and more economical methods to provide shade would be needed. Planting fast-growing trees among the hemlocks may be a more practical and less expensive alternative.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/8/5/156/s1, Table S1: Hemlock woolly adelgid (HWA) density differences of prune, Table S2: Hemlock woolly adelgid (HWA) density differences of shade, Table S3: Hemlock woolly adelgid (HWA) density differences of height, Table S4: Tree health index differences of prune, Table S5: Tree health index differences of shade.

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