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Assessing an integrated biological and chemical control strategy for managing hemlock woolly adelgid in southern Appalachian forests

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ABSTRACT

In the eastern United States, hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) is considered an invasive pest of eastern hemlocks; an ecologically foundational tree species. Current management of HWA focuses on chemical and biological controls, with recent research suggesting that these two tactics could be integrated successfully. The approach is to protect a subset of hemlocks with systemic insecticides while releasing predatory insects onto adjacent, unprotected trees. The goal of this study was to assess the effects of chemical and biological control tactics, alone and in combination, on hemlock health and HWA densities at three southern Appalachian sites (KY, WV, and TN) from 2010 to 2016. Although insecticide applications were effective at protecting individual trees, none of the overall treatments (chemical, biological, or combined) had a significant effect on tree health or HWA population index values relative to untreated plots. Tree health generally declined at all sites over time. HWA populations were highly variable over time and were likely more strongly influenced by extremely low, winter temperatures than by the treatments. Cross-correlation analysis of tree health and HWA population indicated a time-lag effect. At two of the three sites, recovery of tree health lagged 0 – 3 years behind decline in HWA population, and decline in HWA populations lagged approximately 0 – 1 years behind decline in tree health. The predatory beetle, *Laricobius nigrinus*, was recovered two-years, post-release at the KY and WV sites in 2012 and 2013, but was not recovered from the TN site. The lack of sustained recovery of *L. nigrinus* may be attributable to the occurrence of extremely low, winter temperatures in 2014 and 2015, which produced subsequent crashes in the HWA populations. In TN, the *L. nigrinus* population may have been unrecoverable due to a decline in the HWA population shortly after initial release.

1. Introduction

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adeglidae), is found on hemlock species (*Tsuga* spp.) worldwide (Havill and Foottit, 2007). HWA is an obligate herbivore of hemlock trees, which it uses as a secondary host, and spruce species (*Picea* spp.), which are its primary hosts (Havill and Foottit, 2007). In its native ranges, HWA rarely reaches population levels that are injurious to hemlocks because it is kept suppressed through a combination of evolved host resistance and a complex of native predators (Havill et al., 2006; Havill and Foottit, 2007). The insect was discovered in the eastern United States (U.S.) in the early 1950 s near Richmond, VA, and this population has been traced back to its origin in the southern region of the Japanese island of Honshu, near the city of Osaka (Havill et al., 2006; Havill et al., 2014). Since then, HWA has become a serious pest on eastern (*T. canadensis* Carriére) hemlock (Wallace and Hain, 2000; Havill and Foottit, 2007), and is established throughout the eastern U.S. from Maine to Georgia and as far west as Michigan (USFS., 2015).

HWA spreads to trees primarily by wind-blown dispersal, incidental transport by birds and other animals, and via the shipment of infested nursery stock (McClure, 1989b, 1990, Russo et al., 2016). Upon arriving at a hemlock tree, HWA settle and feed at the base of needles. First instars, known as "crawlers", have the capacity to disperse on a host tree and can travel to unoccupied needles (Havill and Foottit, 2007). HWA are bivoltine with a spring generation (progrediens) and a winter generation (sistens). The sistens differ in that they undergo aestival diapause from July through October (Ward et al., 2004; Havill et al., 2014). HWA damages its host by inserting its stylet into the xylem ray parenchyma cells where it feeds (Young et al., 1995). This results in

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carbohydrate depletion, foliar desiccation, and reduction of new growth (Miller-Pierce et al., 2010; Gonda-King et al., 2012; Oten et al., 2012; Domec et al., 2013; Gonda-King et al., 2014). The health of infested hemlock stands is largely dependent upon the density of the HWA (McClure, 1991). Initially, the HWA population causes a decline in tree health. As the trees experience dieback, the HWA population declines due to poor host quality and a lack of new needles on which to settle. This decrease in the HWA population may allow trees to recover and resume new shoot growth. However, new shoots will often be re-infested by HWA and the cycle of decline will continue (McClure, 1991; Orwig et al., 2002). The rate at which this process occurs is not constant. Abiotic factors such as fluctuations in temperature can kill HWA, which can prolong stand survival and slow the range expansion of HWA (Parker et al., 1998, 1999; Skinner et al., 2003; Paradis et al., 2008; McAvoy et al., 2017; Mech et al., 2017).

Eastern hemlock is a long-lived, shade-tolerant, coniferous tree that defines unique ecosystems in eastern North America (Ward et al., 2004), and thus it is considered a foundational species by ecologists (Ellison et al., 2005b, Orwig et al., 2012; Ellison et al., 2016). In areas where hemlock is in decline, major changes have been shown to take place (Ellison et al., 2005a). Stands can transition from hemlock to deciduous hardwoods, and soil-chemical properties can change; namely increased soil-nitrogen content and elevated pH levels (Ellison et al., 2005b). Soil ectomycorrhizal fungi have also been shown to be suppressed in stands experiencing HWA infestation. This can limit future forest regeneration (Lewis et al., 2008). Several avian species have been shown to be closely associated with hemlocks (Tingley et al., 2002). Furthermore, in areas lacking hemlock canopy cover, assemblages of fish and aquatic macroinvertebrates have been shown to be less rich (Evans, 2002).

Management tactics for HWA include chemical and biological controls, precise silvicultural practices, gene conservation, and improvement of host resistance (Vose et al., 2013). A commonly used neonicotinoid insecticide, imidacloprid (Silcox, 2002) is highly effective against HWA (Cowles and Cheah, 2002; Webb et al., 2003; Cowles et al., 2005; Benton et al., 2015). Treatments can be applied in a variety of ways including soil drenches, stem injections, foliar sprays and soil injections (Steward et al., 1998). There are, however, several notable limitations to chemical control programs. First, it is necessary to treat trees individually. Considering the size of a forest, only a small proportion of the trees can reasonably receive treatment. Additionally, the availability of manpower and supplies limits the scale of a chemical treatment program. Finally, the widespread use of insecticides is not desirable because of the potential harmful non-target effects on other insects (Dilling et al., 2009).

Classical biological control programs involve the importation of organisms that possess characteristics that make them able to survive in the targeted environment, unlikely to damage non-target species, and voracious enough to impact the population of the invasive pest. One of the benefits of a biological control program is that the control agent is capable of reproducing and self-dispersing (Van Lenteren et al., 2003). This alleviates a chief limitation of chemical-only controls. Furthermore, a biological control program limits the use of potentially harmful insecticides (Dilling et al., 2009). One of the biological controls for HWA is *Laricobius nigrinus* Fender (Coleoptera: Derodontidae). It is a specialist predator of HWA and can only complete its life cycle on HWA (Zilahi-Balogh et al., 2003b; Zilahi-Balogh et al., 2003a). *L. nigrinus* was cleared for release in 2000 and open releases began in 2003 (Mausel et al., 2010). Since then, > 380,000 individuals have been released in the eastern U.S. with evidence of establishment at many sites (Mausel et al., 2010; Roberts et al., 2011; Davis et al., 2012). *L.* nigrinus has shown the ability to cause significant impact to the winter sistens generation of HWA within four years of release (Mausel et al., 2008; Mayfield et al., 2015). While they can be effective, biological control programs are expensive in terms of the time necessary to identify a potential control agent, perform adequate host-range testing, and establish a field population (Paine et al., 2015). Trees may die before populations of biological control (like *L. nigrinus*) can establish.

The goal of this study was to evaluate an integrated pest management strategy for HWA that combines chemical and biological controls in the same forest stands. Previous research suggests that a combined approach to managing HWA could be effective (Eisenback et al., 2010; Joseph et al., 2011; Mayfield et al., 2015). Under this strategy, chemical treatments could be used to provide initial, temporary protection for a subset of hemlocks in the same forest stand while the *L. nigrinus* population establishes and increases on unprotected trees. After the chemically treated trees lose protection and become re-infested, the *L. nigrinus* population would move onto those trees to provide long-term suppression of HWA. In this study, we attempted to implement this integrated strategy at three sites in the southern Appalachian Mountains. Hemlock tree health, HWA population, and establishment of *L. nigrinus* were monitored over a 5 – 7 year period. We also explored potential correlative relationships between tree health and HWA populations.

2. Methods

2.1. Study sites

The study was conducted at three sites in the southern Appalachian Mountains containing mixed hemlock/deciduous forest. The mean annual minimum winter temperatures were developed from the Plant Hardiness Zones map provided by the United States Department of Agriculture (USDA ARS, 2016). The first site (KY) was established in 2010 at Kentucky Ridge State Forest (36.7196°N, −83.7505°W), with mean annual minimum winter temperatures of −20.6 to −17.8 °C. The second site (WV) was established in 2011 at Twin Falls State Park in West Virginia (37.6221°N, −81.4529°W), with mean annual minimum winter temperatures of −23.3 to −20.6 °C. The third site (TN) was established in 2012 near Oak Ridge, Tennessee (36.0486°N, −84.3879°W) with mean annual minimum winter temperatures of −17.8 to −15.0 °C. Sites were selected based on the widespread abundance of eastern hemlock, presence of HWA throughout the forest, and healthy hemlock crowns at the start of the study. Due to the limited number of *L. nigrinus* beetles that could be acquired for use in any given year, it was necessary to initiate the study at each site sequentially (KY in 2010, WV in 2011, and TN in 2012) rather than simultaneously.

2.2. Assessment of tree health, HWA populations and predator establishment

Tree crown health was evaluated annually every spring using visual estimates of five variables; live crown ratio, live branches, foliage density, new growth and live branch tips. These measurements were rated on a percent scale (0 – 100) by a single observer standing roughly 10 m from the base of each tree. A score closer to 100 represented a healthier tree. The average of these five variables was then used to establish an overall tree crown health index (Jones et al., 2016).

HWA abundance was estimated annually in the spring by randomly sampling the terminal 30 cm of 10 branches from around the entire circumference of the tree. Woolly ovisacs of the current-year sistens were counted on the most recent flush of shoot growth (McClure, 1989a), up to a limit of 20 ovisacs per branch section. One ovisac was considered equivalent to one HWA. The total number of HWA from these branches was then divided by two to arrive at a density index of 0 – 100 (adapted from Cowles et al., 2006). A score closer to 100 represented a more heavily infested tree. This rating method was used because it permits rapid field-assessment and reduces skew from a few number of branches with potentially high population of HWA. Assessment of HWA populations were conducted in the first year of study for each site in order to establish a baseline starting-point for each treatment. The initial HWA index in KY was approximately 30 across all treatments. The initial HWA index in TN was approximately 36, and the initial HWA index for WV was approximately 28. Each of these sites had been infested several years prior to the beginning of the study. US Forest Service county-level records show that reports of HWA began in Wyoming County, WV in 2005, Bell County, KY in 2006, and Morgan County, TN in 2007.

L. nigrinus recovery efforts began two-years post-release at each site. *L. nigrinus* recovery was conducted annually in April and May by beat-sheet sampling for adults and by collecting branches for use in larval rearing (Mausel et al., 2010). All trees were sampled within the treatment plot with the exception of trees that received chemical treatments. Beat-sheet sampling was conducted using a 1 m PVC stick and a rip-stop, nylon sheet approximately 1 × 1 m in size (#2840R BioQuip Products, Inc), for collecting dislodged beetles. Branches for beating were selected from around the entire circumference of each tree. Selection was based upon branch accessibility and a high observed density of HWA. Beating involved 10 – 15 strikes per branch. Any adult beetles that were potentially *Laricobius* (spp.) were collected and taken back to Virginia Tech for identification. The terminal 30 cm of six branches were also clipped from each beetle-release tree. Branches were selected based upon a high density of HWA and branch accessibility on each tree. Branches were collected and taken back to the Virginia Tech Insectary in Blacksburg VA, where they were placed in cages for larval rearing.

Each rearing cage consisted of a Mylar cylinder capped with a polyester mesh cover all affixed to the widest opening of a galvanized metal funnel. The base of the funnel emptied into a 237 ml Mason jar. The cut branches were inserted into water-saturated floral foam and were placed into the rearing cage. The cages were kept at approximately 13 °C (± 2 °C) on a 12:12 h photoperiod to simulate field conditions. Once the larvae finished feeding, they dropped into the Mason jar where they were collected (Salom et al., 2012). The larvae were genetically identified according to Davis et al. (2011). This was necessary due to the presence of the native, *Laricobius rubidus* (LeConte), which can also be found on eastern hemlock trees and which has been found to successfully interbreed with *L. nigrinus* (Zilahi-Balogh et al., 2005; Havill et al., 2012). *L. rubidus, L. nigrinus,* and *L. rubidus x L. nigrinus* hybrid larvae cannot be distinguished morphologically (Havill et al., 2012; Fischer et al., 2015).

2.3. Treatments

At each site, four treatment plots were established and replicated three times for a total of 12 treatment plots. The treatments implemented were (1) chemical, (2) biological, (3) biological + chemical (bio + chem) and (4) untreated control. Plots were defined by the number of trees rather than by area; however, they were often approximately one hectare in size. The primary requirement was that trees from separate plots be no closer than 200 m in order to delay the dispersal of *L. nigrinus* into non-release plots (Davis et al., 2012). Trees were selected on the basis of crown class (about half the trees were suppressed or intermediate and half were codominant), minimal decline in crown health due to HWA (tree health index ≥ 60), and foliage low enough to be sampled using a 5.5 m pole pruner. Tree health index and HWA population index values were recorded for each of the 18 trees in each plot. Of the 18 trees, a subset was selected to receive chemical and/or biological control applications.

2.3.1. Chemical treatment

In each chemical treatment plot, six trees were treated with Merit 2F (imidacloprid, 21.2%), applied at the label rate of 2.33 ml/cm diameter at breast height (DBH). The insecticide was injected approximately 9 cm into the soil at the base of each tree, near the root flare, using a Kioritz© injector (Steward et al., 1998). In 2013, six additional trees were added to each chemical plot at the KY site for a total of 12 treated trees per plot. A similar addition was made to the WV site in 2014, and at the TN site in 2015.

Biological Treatment: In each biological treatment plot, six trees received 125 *L. nigrinus* adults for a total of 750 beetles per plot. All of the beetles used were reared at the Virginia Tech Insectary at Blacksburg, VA and were started from a founding colony of beetles collected in the Seattle, WA area. Beetles were released in KY in 2010, at WV in 2011, and at TN in 2012.

2.3.2. Biological and chemical treatment

In each combined chemical and biological treatment plot, six trees were treated chemically in the same manner as mentioned above and six different trees each received 125 adult *L. nigrinus* for a total of 12 trees receiving treatment. Beginning at the KY site in 2013, six additional trees were treated chemically for a total of 12 chemical and six biologically treated trees. This process was repeated in 2014 at the WV site and in 2015 at the TN site.

2.3.3. Untreated Control

In each plot, all 18 trees received no treatment.

2.4. Statistical analysis

Data for each of the response variables (tree health index and HWA population index) were analyzed by study site using a linear mixed model ANOVA for repeated measures analysis with a first-order autoregressive with random effect covariance structure (Littell et al., 2000; Preisser and Elkinton, 2008; Jones et al., 2016). For each analysis, treatment, year, and the interaction of year∗treatment were the fixed effects factors. Tree nested within treatment and plot was the random effects factor, and year and tree were the repeated measures parameter and subject, respectively. Prior to each analysis, tree health and HWA population index values were tested for normality and, where necessary, were transformed using a Box-Cox or $\log10(y + 1)$ transformation (Zar, 2010). Student's t tests were used for multiple comparisons of the mean responses for significant fixed effects. All mixed model analyses and multiple comparisons were carried out in JMP Pro 12.0.0® (SAS 2013) at a significance level of $\alpha = 0.05$. Graphs were constructed in Microsoft Excel 2016®.

A preliminary time-lag cross-correlation analysis was conducted using the *xcorr* function in MATLAB R2016a (The MathWorks Inc., Natick, MA, USA) to examine the relationship between tree health index values and HWA population index values in the untreated control plots at each of the three study sites. The analyses were set up so that a negative lag time represented the number of years that tree health lagged behind changes in HWA population and a positive lag time represented the number of years HWA population lagged behind changes in tree health. The cross-correlation sequence for each of the study sites was normalized so that the auto-correlation at zero lag was identically 1.0. Following the reasoning of Frost et al. (2013), only normalized cross-correlation coefficients ≥0.5 were considered in determining the time lagged difference between changes in tree health and HWA population at each site.

3. Results

3.1. Kentucky Ridge State Forest, KY

3.1.1. Tree health index

The analysis showed that there was no significant year∗treatment interaction (DF = 18, 34.9; $F = 0.8195$; $p > .05$) or treatment effect (DF = 3, 8.1; $F = 1.8091$; $p > .05$) on mean tree health index at the KY site. Tree health index, however, differed significantly among the seven years that data were recorded (DF = 6, 35.5; $F = 34.5731$; $p < .0001$; Fig. 1A). Mean tree health index in 2010 and 2011 were similar and significantly higher than in other years; the next highest mean tree health index values were recorded in 2012 and 2016, with similar and

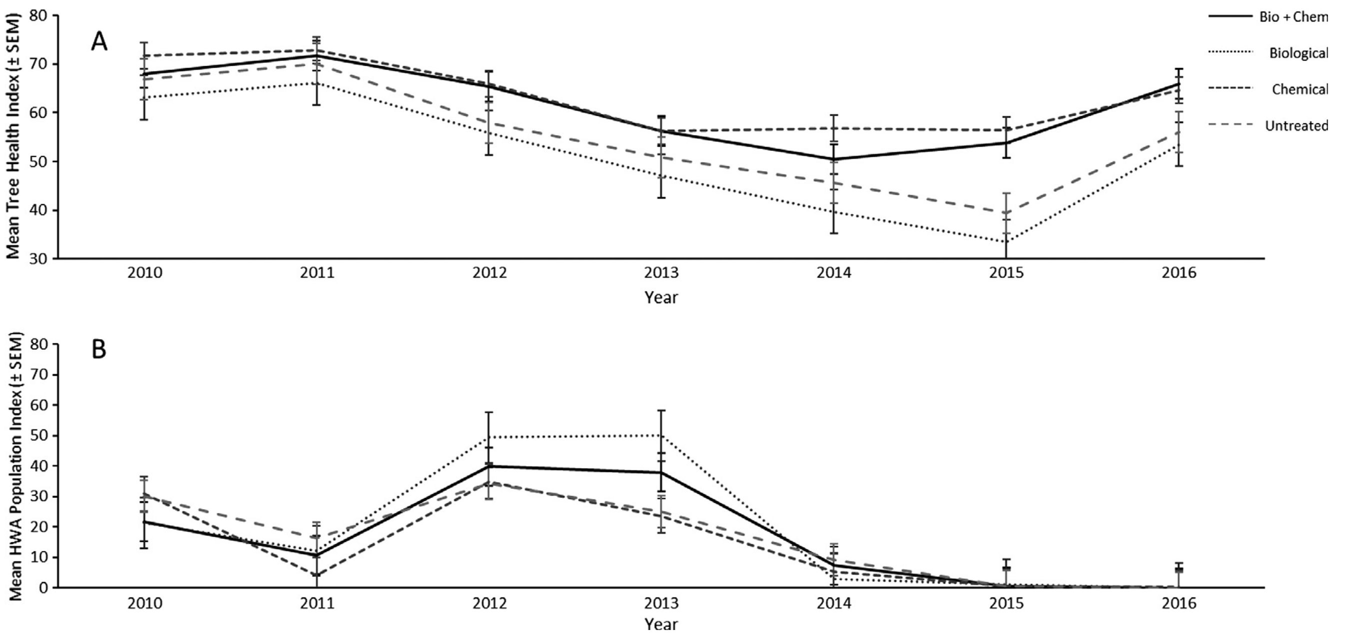

Fig. 1. Hemlock tree health index values (A) and corresponding HWA population index values (B) in biological, chemical, integrated (bio + chem) and untreated control plots from 2010 to 2016 at Kentucky Ridge State Forest, KY.

significantly lower values recorded in 2013, 2014, and 2015 (Fig. 1A). The non-significant year∗treatment interaction indicated that the patterns of tree health index across years were similar for the four treatments, although the pattern for the untreated control plots was consistently lower than for the other treatments (Fig. 1A).

3.1.2. HWA population index

Significant differences in the HWA population index were observed among year (DF = 6, 36.5; $F = 156.8686$; $p < .0001$) at the KY site, but not among treatment (DF = 3, 8.4; $F = 1.2750$; $p > .05$) or treatment over time (DF = 18, 35.6; $F = 1.2728$; $p > .05$; Fig. 1B). Mean HWA population index was statistically similar and highest in 2010, 2012, and 2013 compared with other years. The lowest mean HWA population index was observed in 2016. The patterns of HWA population index changes over time were similar for the four treatments (Fig. 1B).

Examination of the time-lag cross-correlation between tree health index and HWA population index in the untreated controls at the KY site showed that, based on the cutoff in the correlation coefficient (≥0.5), changes in tree health lag approximately 0 – 3 years behind changes in HWA population. Likewise, by the same criterion, changes in HWA populations appear to lag about 0 – 2 years behind changes in tree health index (Table 1).

3.2. Twin Falls State Park, WV

3.2.1. Tree health index

At the WV site, tree health index was significantly affected by year (DF = 5, 27.5; $F = 79.0197$; $p < .0001$), but not by treatment (DF = 3, 8.0; $F = 0.2174$; $p > .05$) or year∗treatment interaction (DF = 15, 27.7; $F = 1.2558$; $p > .05$; Fig. 2A). Tree health index was highest in 2016, followed in deceasing order by 2011 and 2012, then 2013 and 2015, and finally 2014. The non-significant interaction of year∗treatment indicated that the patterns of tree health index changes over time among the four treatments were similar at the WV site.

3.2.2. HWA population index

Year had a significant effect on HWA population index at the WV site (DF = 5, 31.3; $F = 83.3909$; $p < .0001$; Fig. 2B). The effects of year∗treatment (DF = 15, 30.6; $F = 0.8005$; $p > .05$) and treatment (DF = 3, 8.0; $F = 0.0595$; $p > .05$), however, were not significant. Mean HWA population index in 2011, 2012, and 2013 were statistically similar and higher compared to other years. The lowest mean HWA population index was observed in 2016. The patterns of HWA population index changes across year were found to be similar for the four treatments (Fig. 2B).

Table 1
Results of cross-correlation tests of control plots at all study sites. Bolded rows denote strong correlations (≥ 0.50).

KY		WV		TN	
Lag period	Correlation	Lag period	Correlation	Lag period	Correlation
−6	0.21	−5	0.14	−4	0.17
−5	0.26	−4	0.42	−3	0.23
−4	0.48	***−3***	***0.63***	−2	0.35
−3	***0.61***	***−2***	***0.67***	***−1***	***0.53***
−2	***0.69***	***−1***	***0.73***	0	0.88
−1	***0.77***	0	0.78	***1***	***0.66***
0	0.84	***1***	***0.69***	***2***	***0.64***
1	***0.66***	2	0.40	***3***	***0.55***
2	***0.56***	3	0.13	4	0.38
3	0.29	4	0.04		
4	0.08	5	0.002		
5	0.01				
6	0.001				

Examination of the time-lag cross-correlation between tree health index and HWA population index in the untreated controls at the WV site, suggest that changes in tree health lagged approximately 0 – 3 years behind changes in HWA population, and changes in HWA populations lagged approximately 0 – 1 year behind changes in tree health index (Table 1).

3.3. Coal Creek, Tennessee

3.3.1. Tree health index

There was no significant effect of treatment (DF = 3, 10.8; $F = 1.3615$; $p > .05$) or year∗treatment interaction (DF = 12, 32.4; $F = 0.7489$; $p > .05$) on tree health index at the TN site. Tree health index, however, was significantly affected by year (DF = 4, 32.9; $F = 29.1498$; $p < .0001$) with the highest mean index observed in

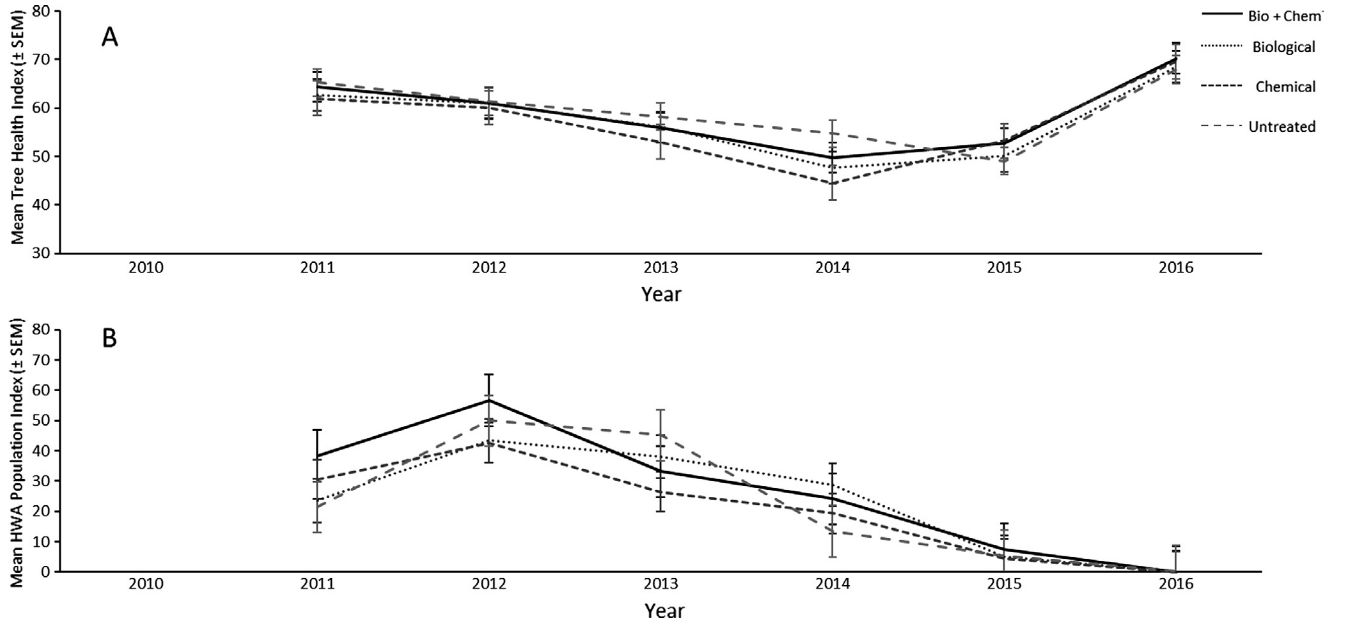

Fig. 2. Hemlock tree health index values (A) and corresponding HWA population index values (B) in biological, chemical, integrated (bio + chem) and untreated control plots from 2010 to 2016 at Twin Falls State Park, WV.

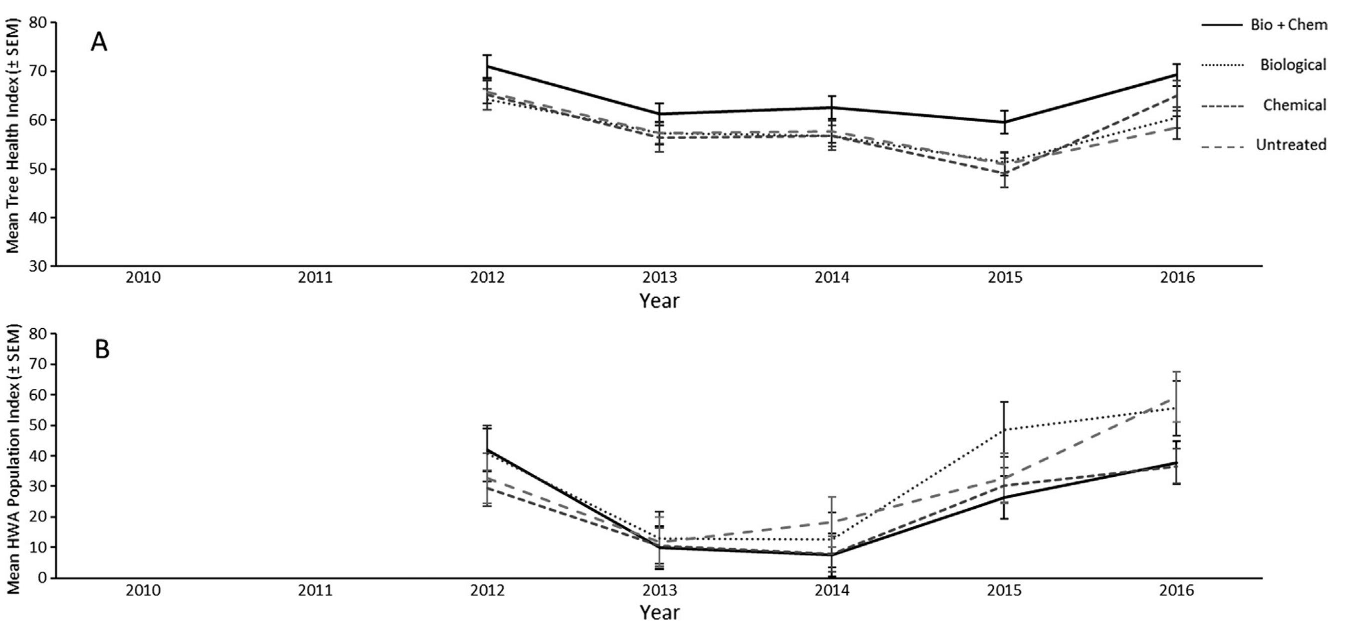

Fig. 3. Hemlock tree health index values (A) and corresponding HWA population index values (B) in biological, chemical, integrated (bio + chem) and untreated control treatments from 2010 to 2016 at Coal Creek, TN.

2012 and 2016, followed by 2014, 2013, and 2015 (Fig. 3A). Similar patterns of tree health index changes over time were observed for the four treatments.

3.3.2. *HWA population index*

Both year (DF = 4, 29.6; $F = 36.3100$; $p < .0001$) and treatment (DF = 3, 15.9; $F = 3.8030$; $p = .0313$) had a significant effect on HWA population index at the TN site. The interaction effect of year∗treatment, however, was not significant (DF = 12, 29.5; $F = 0.9723$; $p > .05$). Mean HWA population index was similar and significantly higher in 2012, 2015, and 2016 compared with 2013 and 2014 (Fig. 3B). Although the analysis showed that the patterns of HWA population index changes across year were similar for the four treatments, the overall mean HWA population index was significantly highest in the biological treatment and lowest in the chemical treatment.

Unlike at the KY and WV sites, examination of the time-lag cross-correlation between tree health index and HWA population index in the untreated controls at the TN site showed that changes in tree health lagged approximately 0 – 1 year behind changes in HWA population; changes in HWA populations lagged about 0 – 3 years behind changes in tree health index (Table 1).

3.4. *Nigrinus recovery*

Adults and larvae (F2 generation) were recovered from two of the three biological replicates and F1 adults and F2 larvae were recovered from all of the bio + chem replicates from the KY site in 2012 (Table 2). Three-years post-release, F3 larvae were recovered from two of the three biological replicates and from one of the three bio + chem replicates at the KY site. No larvae or adults were recovered four to six-

Table 2
Recovery of *L. nigrinus* adults (A) and larvae (L) from KY, WV, and TN released in 2010, 2011, and 2012, respectively.

Site		Years post-release				
Treatment	Replicate	2	3	4	5	6
KY						
Biological	1	1 L	2 L	0	0	0
	2	0	0	0	0	0
	3	1L	9 L	0	0	0
Bio + Chem	1	1 A, 3 L	1 L	0	0	0
	2	9 L	0	0	0	0
	3	1 L	0	0	0	0
WV						
Biological	1	18 L	0	0	0	–
	2	0	0	0	0	–
	3	1 L	0	0	0	–
Bio + Chem	1	0	0	0	0	–
	2	0	0	0	0	–
	3	19 L	0	0	0	–
TN						
Biological	1	0	0	0	–	–
	2	0	0	0	–	–
	3	0	0	0	–	–
Bio + Chem	1	0	0	0	–	–
	2	0	0	0	–	–
	3	0	0	0	–	–

years, post-release. Two-years post-release, F2 larvae were recovered from two of the three biological replicates and from one of the three bio + chem replicates from the WV site in 2013; however, *L. nigrinus* was not recovered three to five years, post-release from 2014 through 2016 (Table 2). No larvae or adults were recovered from the TN site.

4. Discussion

The results of this study indicate that the control methods being used had very-little to no-impact on HWA populations and tree health in the timeframe we tested. Tree health at each site generally declined across all years with evidence of recovery only in 2016. This recovery is likely a result of effects not associated with the treatments. Additionally, HWA populations displayed a great deal of variability and did not show consistent suppression from any treatment type.

Although the efficacy of chemical treatments is well known (Silcox, 2002; Cowles et al., 2006), the chemically treated plots in this study showed declining health and fluctuating HWA numbers. The proportion of trees under chemical protection in each plot began at 33% and was raised to 50%, three years later. This demonstrates that chemical treatments alone, at these proportions, cannot suppress HWA and stabilize tree health. While *L. nigrinus* F2 and F3 generations were recovered from KY, and F2 from WV, no later generations were observed. Confirmation of establishment of *L. nigrinus* was, therefore, not possible at any site. If *L. nigrinus* was present, it may have been so at undetectable densities. This is not to imply that the treatments were entirely ineffective. Plots involving chemical treatments often exhibited reduced HWA populations and greater tree health index values, relative to those that were not chemically treated. Additionally, individual trees that received chemical treatments were often entirely free of HWA and had abundant, healthy foliage. The problem is that these results were not consistent. Frequently, the tree health and/or HWA population index values of chemically treated plots were not significantly different from those of the untreated control or biological treatments. Because plots contained treated and untreated trees together, the positive effects of treatments on plot-level means were diluted by the untreated trees, which continued to deteriorate. Plots were designed this way to simulate a forest management approach wherein it was not feasible to treat every single tree. It was hoped that chemically protecting a subset of hemlocks would be sufficient to maintain or improve stand health while the biological control established. This aligns with one of the goals of IPM by reducing the use of chemical insecticides (Ehler, 2006). The purpose of the biological control is to overcome the limitations of chemical treatments, and to act on its own to target and eliminate HWA. Ultimately, the lack of consistency indicates that other factors may have had greater impacts on tree health and HWA population than the treatment methods.

There are behavioral and physiological responses to inter-species competition. Often such responses do not follow a strict chronological order (Brooks et al., 1999; Metzger et al., 2009). The interaction of HWA and eastern hemlock is one such example. McClure (1991) showed that the progress of an HWA invasion is intimately linked with the health of the trees on which they feed. His study showed that HWA populations peaked in the first year of colonization. In the second year, HWA populations declined due to the reduced health of the trees, and tree health consequently improved. Then, in the third year, the HWA densities recovered on the new foliage of the partially-recovered hemlocks. By the fourth year, the trees were again in severe decline. The results of our study illustrate this type of density-dependent feedback between host quality and HWA density. Results of the cross-correlation analysis show that at the KY site changes in tree health lagged up to three years behind changes in the HWA population. This conclusion was reached based on the strong correlation present in the analysis at lag periods −3, −2, and −1. Changes in the HWA population lagged 0 – 2 years behind changes in tree health. Tree health at the KY site began to fall after 2011, and HWA populations did not begin to decline until after 2012. This was followed by a steady decline in tree health and HWA population until 2015, when tree health began to recover. The HWA population had not begun to recover by the end of the study. A similar pattern was observed at the WV site. There, tree health lagged 0 – 3 years behind changes in the HWA population, and the HWA population lagged 0 – 1 year behind changes in tree health. The results differed at the TN site. There, tree health seemed to be strongly correlated only 0 – 1 years behind changes in HWA population, and HWA populations showed strong correlations Null – 3 years after changes in tree health. This difference could be due to the shorter period of study compared to KY or WV, which may have missed changes in the HWA population prior to 2012. It could also be due to the fact that the TN site occupies a warmer climate than KY or WV. Our results differ from the patterns described in McClure (1991) in that the time lags were longer in the current study. In McClure's (1991) experiment, it took four years until all of his stands were in severe decline; however, some infested hemlock stands may survive for more than a decade (Eschtruth et al., 2013). This implies that the rate of decline of a hemlock stand can be highly variable. The fact that tree health at some sites took up to three years to reflect changes in HWA density could be due to long-term physiological responses to HWA feeding. As mentioned previously, HWA can reduce new growth, deplete photosynthate, and cause the formation of false growth-rings (Gonda-King et al., 2012; Oten et al., 2012). Each of these outcomes has an impact on the trees' ability to sequester nutrients and to maintain efficient transportation of water and food, which may delay recovery and produce variable lag periods. Describing hemlock and HWA interactions in the context of a time-lagged correlation is a novel means of interpretation and one that lends itself well to studying complex ecosystem processes (Loehle and Li, 1996; Metzger et al., 2009). Continued observation is needed at each site in order to gain a better understanding of the yearly flux of HWA and tree health.

Although we did not intentionally focus on weather, its potential influence on the outcome of the study warrants consideration because cold winter weather presents a limit to the range expansion and establishment of HWA (Costa et al., 2008; Trotter and Shields, 2009; Trotter, 2010). The occurrence of extremely cold winter temperatures in 2014 and 2015 led to significant HWA mortality across the range of hemlock in the eastern U.S. (McAvoy et al., 2017; Tobin et al., 2017).

Winter mortality of HWA may be a function of several factors, including the lowest minimum temperature reached, the number of days with minimum temperatures below a threshold (e.g., −1 °C), and mean temperatures immediately prior to a cold event (McAvoy et al., 2017). Cold hardiness in HWA can be induced through prolonged exposure to cold temperatures (Elkinton et al., 2016), suggesting rapid temperature reductions may be particularly important in causing winter HWA mortality. Like much of the eastern U.S., our study sites in KY and WV experienced extreme cold weather events in January 2014 (KY: −19.4 °C, WV: −22.8 °C) and February 2015 (KY: −23.3 °C, WV: −26.1 °C), and these sites showed subsequent HWA decline in those years (Figs. 1 and 2). The comparatively warmer weather in TN and the lower minimum temperatures at this site during the same period (2014: −17.2 °C, 2015: −18.3 °C) likely caused or contributed to the lack of an HWA population crash at that site (Fig. 3)

In hopes of ensuring establishment of *L. nigrinus*, each release plot received approximately 750 adult beetles (125 per tree × 6 trees). Based on these release sizes, the mean winter temperature at the sites, and the predictive model presented by Mausel et al. (2010), the likelihood of *L. nigrinus* establishing at sites KY and TN was estimated to be 85 – 95%, and approximately 60 – 85% at site WV. However, there was a lack of sustained recovery of *L. nigrinus* despite the large, initial introductions. This was likely due in large part to the extremely-cold winter temperatures that occurred in 2014 and 2015 and the associated decline in available adelgid prey. Coincidentally, recovery of *L. nigrinus* failed in these years continuing through 2016. One may conclude from these data that the crash in the HWA population produced a concurrent decline in *L. nigrinus*. However, because *L. nigrinus* was successfully recovered several-years prior, a remnant population might remain. At the TN site, *L. nigrinus* was introduced in 2012 and recovery attempts began in 2014. After the releases, the HWA population declined in 2013 and remained low in 2014. It is possible that *L. nigrinus* established, albeit at low densities. Continued monitoring of these sites needs will be necessary to determine whether a population of *L. nigrinus* persists and can rebound.

Beat-sheet sampling was used in this study to recover adult *L. nigrinus*. Beat-sheet sampling is often used to survey a large study area due to the speed with which vegetation can be surveyed; however, it has been criticized for creating false negatives (Mausel et al., 2010). This is due to the fact that a researcher may miss the presence of *L. nigrinus* either due to the poor timing of surveys or inability to reach portions of the trees where the adults are located. In order to partially-mitigate this shortcoming, larvae were also sampled by collecting branches using pole pruners. This technique targets a less mobile life stage, enables sampling higher in the canopy, and directly targets large concentrations of HWA, where *L. nigrinus* larvae are likely to be feeding. Unfortunately, no larvae were recovered after 2013 using this method, indicating that the *L. nigrinus* population was too low to be detected.

While the treatment methods did not achieve consistent improvement in plot-level hemlock health as we anticipated, this study did document area-wide changes in eastern hemlock forest health and HWA populations over time. We showed that HWA infestations are variable in severity and that substantial improvements in tree health can occur after HWA populations decline, even as many as 10 years after initial infestation (e.g. sites KY and WV, Figs. 1 and 2). Additionally, our investigation into the time-lag relationship of tree health and HWA population may help forest health professionals explain why hemlock stands do not immediately respond to HWA suppression efforts. This information should be kept in mind by forest managers when selecting sites for treatment. The delayed effect of HWA feeding on tree health means that healthy stands (such as a tree health index ≥60 using this method) should be selected for treatment before they become badly damaged (health ≤20). Otherwise, suppression efforts may be too late to save many trees.

The results of this study do not negate the potential use of an integrated chemical and biological control strategy for managing eastern hemlock. Rather, they illustrate the potential fragility of the biological control component, and the importance of an established and persistent predator population if the strategy is to work. Our study also illustrates the importance of regular surveys to determine the survival and impact of *L. nigrinus*, and the potential need to augment initial releases with additional beetles. Such augmentative releases may be particularly important following extreme cold weather events that drastically reduce HWA populations, but should be delayed until adelgid prey are present again. Future research could consider varying the proportion of chemically treated and untreated trees in the stand, as well as the type, rate, and timing of the chemical treatment, so that different cohorts of protected trees become unprotected (and thus eligible to support predators again) at different times. Finally, due to the highly variable nature of tree health, adelgid density and predator abundance from year to year, long-term field studies of greater duration than this one are likely necessary to adequately assess the efficacy of an integrated chemical and biological control strategy.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2018.01.018.

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