Entomologia
Experimentalis et Applicata

DOI: 10.1111/eea.12343

15TH INTERNATIONAL SYMPOSIUM ON INSECT-PLANT RELATIONSHIPS

Contrasting effects of two exotic invasive hemipterans on whole-plant resource allocation in a declining conifer

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Accepted: 22 June 2015

Key words: *Fiorinia externa*, *Adelges tsugae*, *Tsuga canadensis*, resource partitioning, starch, protein, Hemiptera, Adelgidae, Diaspididae

Abstract

Invasive herbivores can cause widespread dieback of naïve native hosts in the invaded range. Some consume leaves, some bore through wood, whereas others, such as piercing-sucking insects, alter plant resource allocation through changes to source-sink dynamics and depletion of long-term stores. Invasive sap-sucking herbivores that target cells critical to resource transport and storage may have particularly large effects. Herbivory by two exotic hemipterans, hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Adelgidae), and elongate hemlock scale (EHS), *Fiorinia externa* Ferris (Diaspididae), have very different effects on eastern hemlock, *Tsuga canadensis* (L.) Carrière (Pinaceae). Although these insects differ in both timing and feeding site on their hemlock host, the reasons for their differential effects are poorly understood. Here, using potted seedlings in a common garden, we examined the effects of these two herbivores on resource uptake and allocation immediately after an initial attack. We labeled the plants with a single pulse of $^{13}CO_2$ and a supply of $^{15}NH_4{}^{15}NO_3$ every third day to obtain a whole-plant perspective on resource uptake and allocation. After 10 weeks of controlled infestation, plants were measured and divided into tissue types (needles, branches, main stem, and roots). In each tissue we quantified biomass, ^{13}C, ^{15}N, total carbon (C), nitrogen (N), protein, and starch pools. Hemlock woolly adelgid feeding decreased new needle biomass by 34%, increased ^{13}C allocation to roots and main stems by 130%, and increased ^{15}N allocation to old foliage by 18%. Hemlock woolly adelgid infestation also resulted in increased starch storage in old branches over new needles, and marginally increased protein content plant-wide. Elongate hemlock scale infestation resulted in a different growth pattern, with a 27% increase in biomass allocation to the main stem. Elongate hemlock scale also caused a 23% increase in N allocation to roots and main stem. Increases in resource allocation to main stem and belowground may indicate herbivore-induced changes to storage patterns, or compensatory increased fine root growth to facilitate nutrient and water uptake. These resource allocation effects likely underlie the rapid and dramatic decline of hemlock in response to HWA feeding, and the considerably milder effects of EHS feeding.

Introduction

Herbivores have strong effects on the physiology, ecology, and evolution of plants in both agricultural and natural systems (Fritz & Simms, 1992; Baldwin & Preston, 1999; Strauss & Agrawal, 1999). Non-native invasive herbivores can have particularly large effects on naïve, poorly defended resident hosts (Herms & Mattson, 1992; Gandhi & Herms, 2010; Desurmont et al., 2011). In North America, for example, the emerald ash borer, *Agrilus planipennis* Fairmaire, is causing widespread dieback of ash (Poland & McCullough, 2006), and the Asian longhorn beetle, *Anoplophora glabripennis* (Motschulsky), is causing extensive mortality of maples (*Acer* spp.), willows (*Salix* spp.), and elms (*Ulmus* spp.) (Nowak et al., 2001; Smith et al., 2002). In addition to these insects, invasive sap-sucking

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herbivores that feed on cells and tissues critical to resource transport and storage can have devastating effects on plant growth, reproduction, and survival (Meyer & Root, 1996; Kaloshian & Walling, 2005; Walling, 2008).

Herbivores can directly limit resource availability in the host if there is loss of photosynthetic area or fine root biomass (Wise & Abrahamson, 2007). Insects can also have indirect effects on plant growth and reproduction through herbivore-induced changes to resource dynamics, both locally and systemically (Strauss & Agrawal, 1999; Karban & Baldwin, 2007; Orians et al., 2011; Schultz et al., 2013). Many insects, for example, can co-opt plant resources for their own benefit (Gómez et al., 2012; Rehill & Schultz, 2012).

In some cases, herbivore-induced changes in resource allocation to storage tissues may provide some benefit to the plant (e.g., induced sequestration, sensu Orians et al., 2011). Frost & Hunter (2008), for example, found greater allocation belowground following herbivory. Induced sequestration of resources away from attacked sites is hypothesized as an induced tolerance strategy against leaf chewing and defoliating herbivores (Kaplan et al., 2008; Gómez et al., 2010; Schultz et al., 2013; Korpita et al., 2014), and likely favored in environments with high herbivore densities (Heil, 2010). Reallocation of phloem-transported resources can also be a mechanism to provide resources for the induction of plant defense traits (Schultz et al., 2013). These shifts in allocation are expected to come at the cost of reduced growth and reproduction and/or increased susceptibility to environmental stress (Schultz et al., 2013) due to plant functional trade-offs (Herms & Mattson, 1992).

Overall, evergreen species may be particularly sensitive to damage or to induced changes in resource dynamics (Nykänen & Koricheva, 2004). Compared with deciduous species, which store C and N in inaccessible tissues such as stems and roots (Hoch et al., 2003), evergreen species rely more heavily on foliar storage of C (Bryant et al., 1983; but see Vanderklein & Reich, 2000). In addition, evergreen species invest more N in leaf persistence than do deciduous species (Takashima et al., 2004). Understory seedlings are expected to be particularly sensitive to attack. Low light availability limits C availability (Canham et al., 1994), and the slow rate of decomposition, mineralization, and nitrification in conifer-dominated systems can make N more limiting (McClaugherty et al., 1985).

Eastern hemlock, *Tsuga canadensis* (L.) Carrière (Pinaceae), a conifer in eastern North America, is under attack by several invasive species (Gómez et al., 2015). One of these exotic herbivores, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a specialist responsible for widespread mortality of eastern hemlock in the eastern USA (Orwig et al., 2002). Hemlock woolly adelgid has two generations per year and feeds on parenchyma cells at the base of the needles. A second common exotic hemipteran, the elongate hemlock scale (EHS), *Fiorinia externa* Ferris (Diaspididae), only causes mortality in severely stressed trees (McClure, 1980). Elongate hemlock scale has one generation per year and feeds on the mesophyll of needles themselves. Marked differences in feeding site and phenology may contribute to differing effects on resource allocation, and thus the severity in host damage.

Upon infestation with HWA, the long-lived *T. canadensis* suffers reduced photosynthesis, reduced growth, increased needle loss, and branch fall, leading to rapid mortality (Broeckling & Salom, 2003; Stadler et al., 2005; Nuckolls et al., 2009; Gonda-King et al., 2014). This rapid physiological response may indicate resource manipulation by the insect, followed by depletion. In its native range, HWA forms galls on spruce, its primary host (Havill et al., 2007), suggesting that even though it does not form galls in eastern hemlock it may retain some capacity to alter host physiology. Galling species can upregulate primary metabolism by manipulating sink strength of the attacked tissues (Larson & Whitham, 1991). In addition, Gómez et al. (2012) observed higher free amino acid concentrations in HWA-infested tissues of *T. canadensis* compared with tissues in noninfested trees, a result consistent with resource manipulation. Moreover, in contrast to HWA, amino acid concentrations in EHS-infested tissues were not affected compared with control plants (Gómez et al., 2012). This suggests that EHS may not affect resource allocation. Hemlock woolly adelgid has two generations per year, with the first generation coinciding with peak plant growth, whereas EHS has a single generation. When EHS and HWA co-occur on *T. canadensis*, many of the symptoms expected under HWA infestation are alleviated (Miller-Pierce et al., 2010).

How HWA and EHS differ in their effects during early stages of infestation is not well-studied and is likely an important factor in determining their effects on host survival. Here, we examined the individual effects of these two herbivores on resource uptake and whole-plant resource allocation. More specifically, we measured biomass growth, C and N concentration, distribution of C and N stable-isotope labels, and storage (starch and protein concentration) in the first season of HWA and EHS infection. We performed infestation treatments on 1-year-old seedlings in a shaded plot, to differentiate the effects of each insect from the physiology of healthy control plants. We used the natural attack phenology rather than trying to manipulate the two insects to synchronize their timing of attack.

We hypothesized that HWA disrupts the ability of hemlocks to effectively uptake and invest C and N sources to growth and storage. Reduced fine root production and needle growth would contribute to this effect. We also expected to observe signs of resource depletion, manifested as decreases in starch storage and in new growth. We hypothesized that HWA-infested trees increase protein breakdown for N reallocation, potentially contributing to previously observed increases in the amino acid content of infested foliage. Given the milder effects of EHS on hemlock growth and mortality, we hypothesized that EHS herbivory would have minimal effects on resource uptake and allocation compared with HWA.

Materials and methods

Study system

Hemlock woolly adelgid was most likely first introduced from southern Japan to the USA in the 1950s (Ward et al., 2004; Havill et al., 2006), and has since spread throughout the range of *T. canadensis* and *T. caroliniana*; from the Carolinas and Tennessee in the south to near the northern border of Massachusetts (Morin et al., 2009). In its native range across Asia, HWA feeds on *Tsuga* and *Picea* species in alternating generations; in its introduced range it relies only on *Tsuga* hosts (McClure, 1989). Hemlock woolly adelgid settles at the base of *T. canadensis* needles and inserts its stylet bundle to extract water and nutrients from the xylem ray parenchyma of the stem (Young et al., 1995), likely impacting nutrient transport and storage (van Bel, 1990). The spring generation of HWA emerges at the end of April and immediately begins feeding before bud-break; a second generation of HWA emerges mid-summer.

Elongate hemlock scale is also native to Asia, and was introduced to New York (USA) in 1908 (Sasscer, 1912). It began to invade in the 1970s to encompass its current range of 14 eastern states, largely overlapping with the range of HWA (Lambdin et al., 2005). Elongate hemlock scale is also widespread throughout the range of *T. canadensis* (Preisser et al., 2008). It feeds on the underside of needles, sucking from the leaf mesophyll (McClure, 2002). In the northeastern USA, EHS emerges later in the season (late June), close to the end of eastern hemlock's annual growth.

Eastern hemlock is common in forests throughout the eastern USA and Canada. It is a shade-tolerant and late-successional species that often occurs in dense stands (Orwig & Foster, 1998). *Tsuga canadensis* has virtually no natural resistance to HWA (McClure, 1995; Orwig & Foster, 1998; but see Preisser et al., 2011).

Experimental design

To assess whole-tree effects of herbivory, we used 1-year-old *T. canadensis* seedlings from Evergreen Nursery (Sturgeon Bay, WI, USA). Fifty-two seedlings were potted and placed in a shaded garden plot on Tufts University campus (42.41°N, 71.12°W) (Medford, MA, USA), within the current ranges of all three species, and watered as needed. Because HWA and EHS have different phenology of feeding activity, we randomly split our trees into two experiments to match the developmental timing of each insect. Each insect experiment included a randomly selected control group of noninfested trees. Two randomly selected trees from each control group were designated as unlabeled controls to determine the natural abundance of ^{13}C and ^{15}N before isotope application. One control tree in the EHS experiment was added a posteriori to the natural abundance subset for C analysis due to failure of ^{13}C labeling. This resulted in the following allocation of seedlings per treatment: 13 HWA-infested, 13 HWA-control (two isotopically unlabeled), 13 EHS-infested, 12 EHS-control (two ^{15}N and ^{13}C unlabeled, one ^{13}C unlabeled).

Herbivory treatment

Each tree received insect-infested inoculant branches according to standard protocols (Miller-Pierce et al., 2010) allowing crawlers to colonize the entire trees. All trees were bagged to prevent insect-contamination across treatments and experiments. Both insects are sessile and differ in the time window in which the mobile juvenile instars are available; HWA in late April and EHS in late June. To best incorporate natural differences in the phenology of attack, we added the insect treatments on dates of first crawler emergence for each species. In April 2012, immediately before the first HWA emergence, inoculant branches (8–10 cm) naturally infested with HWA were collected in Medford, MA, USA (42.44°N, 71.12°W) and attached to trees in the HWA group. In order to control for the effects of applying foliage to the plants, noninfested foliage from the same field site was attached to trees in the control treatments. We reinoculated 2 weeks later to assure dense HWA settlement. In late May, before EHS emergence, naturally infested foliage was collected from Hyde Park in Stafford, CT, USA (41.95°N, 72.31°W) and applied to the EHS trees and their control group, respectively. We performed two additional EHS inoculations in June and July, until we observed EHS settling along the new growth of the inoculated trees.

Isotope labeling

All plants received 50 ml $^{15}NH_4{}^{15}NO_3$ (50 p.p.m.) every third day beginning mid-May, for a total of 8 weeks prior to HWA harvest, and 19 weeks prior to EHS harvest (beginning with the May inoculation). Unlabeled control seedlings received only water over this period. We saw no evidence of a fertilization effect in the growth comparisons between natural abundance (unlabeled) seedlings and labeled control seedlings in either the HWA experiment (Bonferroni-adjusted t-tests of tissue mass, with lowest P-value for roots: t = 2.63, d.f. = 11, P = 0.14) or EHS experiment (P = 1.0 for all). Six days before plant harvest, we applied $^{13}CO_2$ to each tree on a sunny morning to conduct a pulse-chase experiment in photosynthate. We exposed one primary branch in the upper portion of each tree to $^{13}CO_2$ by placing the branch into a sealed plastic sleeve and injecting 20% lactic acid solution into a 20-mg vial of 99% ^{13}C sodium bicarbonate, producing a $^{13}CO_2$ pulse of up to 0.24 mol (as in Bledsoe & Orians, 2006).

Plant harvest

One day prior to harvest, we measured (1) branch growth, as length of terminal current-year growth on three randomly selected branches per seedling, and (2) insect infestation density. During the harvest, we washed soil from the roots, then separated the plant into seven tissue categories: roots (R), main stem (S), new growth [branch (NB) and needle (NN) separated], previous years' growth [branch (OB) and needle (ON) separated], and the branch directly exposed to $^{13}CO_2$ (LG). We flash-froze a 5-g subsample from each tissue category in liquid nitrogen (N), lyophilized to constant mass and stored at −20 °C for further chemical analysis. The remaining tissue was oven-dried (75 °C) to constant mass and weighed.

Resource transport and allocation

Stable isotopes and C:N. Each tissue was ground to a fine powder using a ball mill (Kleco, Visalia, CA, USA). A small amount (1–5 mg) of each tissue sample was sent to the University of California-Davis Stable Isotope Facility for ^{13}C:^{12}C and ^{15}N:^{14}N isotope ratio mass spectrometry as well as measurement of total C and N concentration. The isotopic composition of each sample was reported in δ notation as permil (parts per thousand), relative to standards of known composition (peach leaves, nylon, USGS-41 glutamic acid). In addition, we converted absolute ^{13}C and ^{15}N measurements to net values in excess of natural abundance levels prior to statistical analysis. Lower ^{13}C values are observed and expected in comparisons of spring to summer (HWA vs. EHS experiments), as natural abundance ^{13}C shifts toward depletion in conifer needles over the course of the growth season (Jäggi et al., 2002).

Starch and protein concentration. Starch was measured using standard techniques (Haissig & Dickson, 1979). Ten milligram of each tissue type was extracted in 80% ethanol to remove ethanol-soluble compounds, and the resultant pellets were analyzed for starch concentration, an important storage compound in conifers (Webb, 1981). The pellets were digested overnight in an amyloglucosidase and sodium acetate solution at 55 °C (Haissig & Dickson, 1979), and the resulting free sugars were analyzed. We measured absorbance by the phenol-sulfuric acid method (Dubois et al., 1956) at 487 nm using a microtiter plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Protein concentration in the different tissues was quantified using a colorimetric method (Bradford & Williams, 1976). Ten milligram of ground tissue was extracted in 1.5 ml of NaOH 0.1 N. The extracts were incubated at 100 °C for 2 h and a dye-binding reagent (#500-0006; Bio-Rad) was used to quantify protein based on sample absorbance at 595 nm using bovine serum albumin as a standard.

Statistical analysis

Due to deviations from normality and homoscedasticity across all variables measured, we rank-transformed data prior to analysis. We analyzed the transformed data using linear mixed models implemented in R (nlme: Pinheiro et al., 2007), including insect treatment as a fixed effect. Because cellular physiology is highly differentiated by tissue in the plant, we included tissue type as a fixed effect. We also included the interaction term between tissue and insect treatment in the model. To eliminate pseudoreplication due to the analysis of six tissue types per tree, we included individual tree identity as a random effect in the mixed-effects model. Due to small sample size, we did not include additional covariates in our analysis.

Results

Infestation level

Our artificial infestations resulted in 1.88 ± 0.24 and 0.98 ± 0.18 insects cm^{-1} new growth (mean ± SE) for HWA- and EHS-treated plants, respectively, at the times of their respective harvest. In addition, EHS infested the old foliage throughout the plant, so our measured plant-wide densities were conservatively low.

Plant growth

After only 10 weeks of infestation from the initial inoculation date, HWA already reduced average branch elongation (t-test: t = 2.45, d.f. = 20, P = 0.045) by approximately one-third compared with control trees (Figure 1A). Biomass allocation was significantly affected by the interaction between HWA treatment and tissue type

(Table 1, Figure 2A); HWA infestation most strongly decreased biomass of new foliage (needles and branches), simultaneously increasing relative biomass of old foliage. In contrast, 10 weeks of EHS infestation from the last inoculation date did not result in any change in branch elongation (t-test: t = 0.96, d.f. = 20, P = 0.69), though an interaction between EHS treatment and tissue type significantly affected relative biomass allocation (Table 2, Figures 1B and 2B). In contrast to the patterns observed in HWA-infested trees, EHS-treated plants exhibited an increase in the biomass of the main stem, with a concurrent decrease in the biomass of old needles.

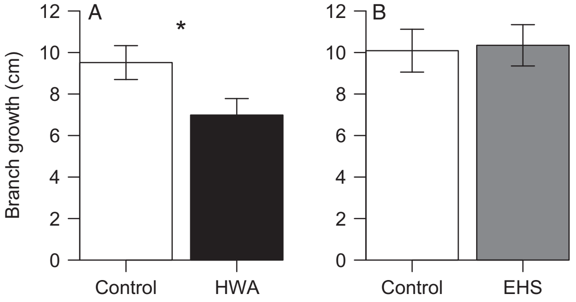

Figure 1 Mean (± SE) eastern hemlock growth (cm) in response to herbivory by (A) hemlock woolly adelgid (HWA) and (B) elongate hemlock scale (EHS). New branch growth is quantified as length of new growth (elongation) for a subset of three longest branches per tree (t-test: *P<0.05).

Resource transport and allocation

Carbon and nitrogen uptake and allocation. Neither insect had a net effect on total C or N, or on C:N ratio (Tables 1 and 2, Figure 3). Compared with the HWA experiment, plants in the EHS experiment exhibited less C uptake overall. Across the EHS experiment insect treatments, $\delta^{13}C$ was more negative (indicating less uptake). An interaction between HWA infestation and tissue type significantly affected ^{13}C and ^{15}N translocation (Table 1, Figure 4A and C). Hemlock woolly adelgid plants translocated more ^{13}C toward roots and main stems, and more ^{15}N toward old tissue (needles and branches) where they were feeding, with a corresponding decrease in new needles and the main stem. In contrast, the effects of EHS on resource allocation were minimal. Elongate hemlock

Table 1 Results of mixed model assessing the effects of hemlock woolly adelgid infestation on resource allocation and resource pools. We examined tissue and herbivory effects and their interaction. All data were rank-transformed prior to analysis

	Tissue		Herbivory		Tissue*herbivory	
	$F_{5,110}$	P	$F_{1,22}$	P	$F_{5,110}$	P
$\delta^{13}C$	39.3	<0.0001	0.923	0.35	3.17	0.010
$\delta^{15}N$	89.9	<0.0001	0.198	0.66	2.63	0.028
Starch	1.30	0.27	0.526	0.48	5.14	0.0003
Protein	56.6	<0.0001	3.55	0.073	1.29	0.28
C:N	221	<0.0001	2.55	0.13	10.9	0.37
%C	31.7	<0.0001	2.81	0.11	0.731	0.60
%N	220	<0.0001	2.58	0.12	1.11	0.36
Biomass	110	<0.0001	0.0122	0.91	15.1	<0.0001

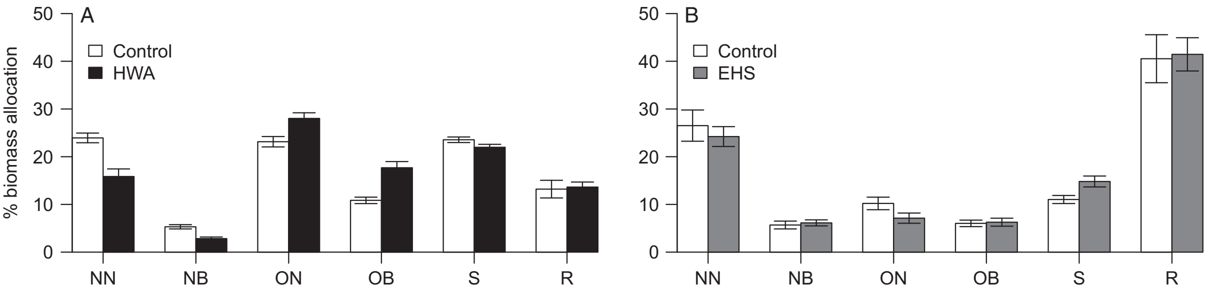

Figure 2 Mean (± SE) biomass allocation (%) to new needles (NN), new branches (NB), old needles (ON), old branches (OB), main stem (S), and roots (R) of eastern hemlock in response to herbivory by (A) hemlock woolly adelgid (HWA) and (B) elongate hemlock scale (EHS).

Table 2 Results of mixed model assessing the effects of elongate hemlock scale (EHS) infestation on resource allocation and resource pools. We examined tissue and herbivory effects and their interaction. All data were rank-transformed prior to analysis. New needles and new branches compare control to locally EHS-infested tissues only

	Tissue				Herbivory				Tissue*herbivory			
	Num d.f.	Den d.f.	F	P	Num d.f.	Den d.f.	F	P	Num d.f.	Den d.f.	F	P
$\delta^{13}C$	5	100	32.2	<0.0001	1	20	0.313	0.58	5	100	0.6719	0.65
$\delta^{15}N$	5	105	115	<0.0001	1	21	0.255	0.62	5	105	2.1419	0.066
Starch	5	100	30.1	<0.0001	1	20	0.227	0.64	5	100	0.7558	0.58
Protein	5	105	6.70	<0.0001	1	21	0.215	0.65	5	105	1.406	0.23
C:N	5	106	214	<0.0001	1	20	0.0178	0.90	5	106	0.6803	0.64
%C	5	101	39.0	<0.0001	1	19	1.28	0.30	5	101	1.1953	0.30
%N	5	106	181	<0.0001	1	20	0.136	0.72	5	106	0.8789	0.50
Biomass	5	100	98.2	<0.0001	1	20	0.0261	0.87	5	100	2.6142	0.029

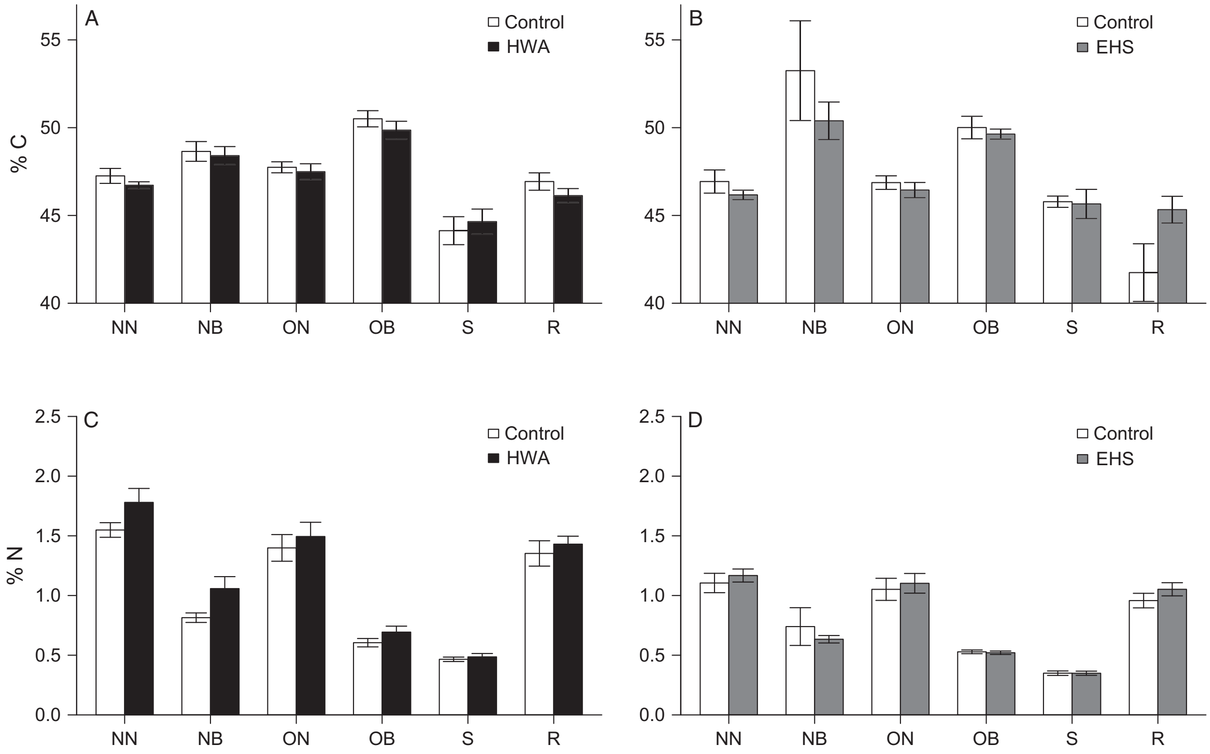

Figure 3 Mean (± SE) percentage of C and N in new needles (NN), new branches (NB), old needles (ON), old branches(OB), main stem (S), and roots (R) of eastern hemlock in response to herbivory by (A, C) hemlock woolly adelgid (HWA) and (B, D) elongate hemlock scale (EHS). In EHS trees, new needles (NN) and new branches (NB) represent only locally infested tissues.

scale had no effect on ^{13}C translocation (Table 2, Figure 4B). For ^{15}N, an interaction between EHS infestation and tissue type had a marginally significant effect on ^{15}N translocation (Table 2, Figure 4D); EHS increased translocation to the roots and main stem.

Starch and protein concentrations. The interaction between HWA treatment and tissue had a highly significant effect on starch concentration (Table 1, Figure 5A), but neither EHS infestation nor its interaction effects significantly altered starch concentration (Table 2, Figure 5B). Hemlock woolly adelgid induced an elevation in starch concentrations in old branches, but a decrease in starch concentrations in new needles. A marginally significant elevation in protein concentration was observed for HWA-infested plants (Table 1, Figure 5C),

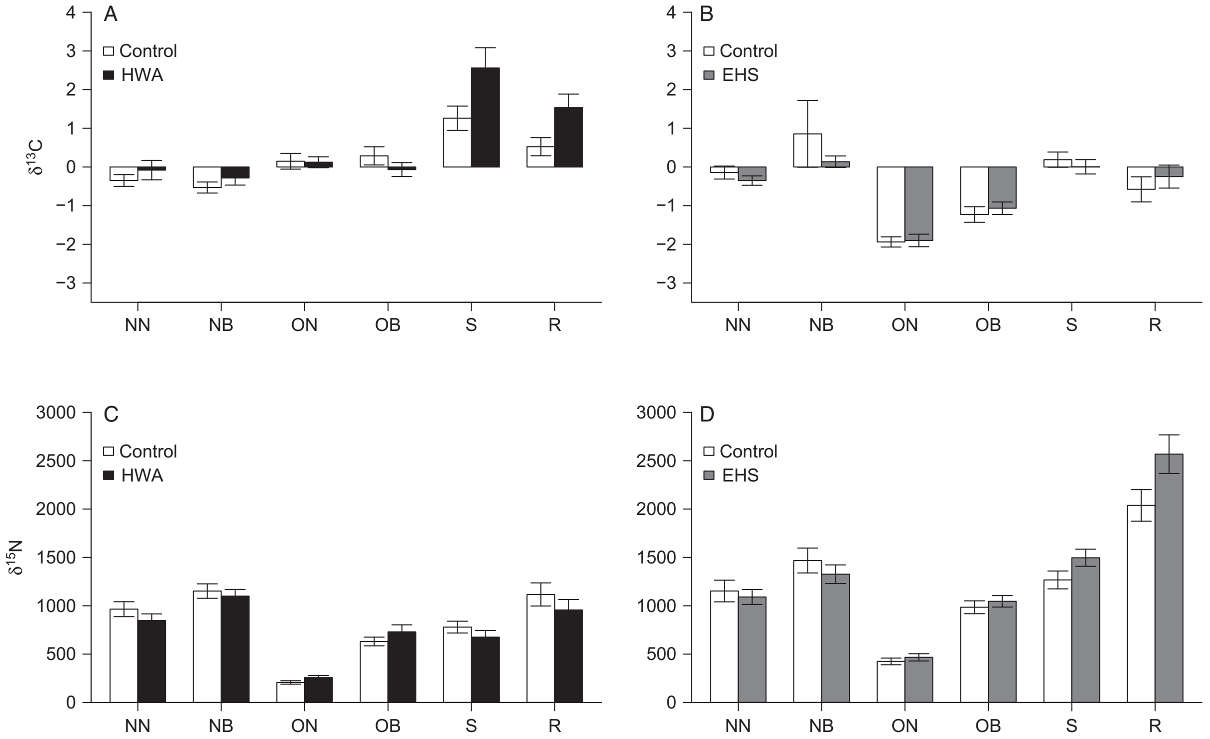

Figure 4 Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ isotope in excess of natural background in new needles (NN), new branches (NB), old needles (ON), old branches (OB), main stem (S), and roots (R) of eastern hemlock in response to herbivory by (A, C) hemlock woolly adelgid (HWA) and (B, D) elongate hemlock scale (EHS). Natural abundance background values were subtracted for each value. In EHS trees, new needles (NN) and new branches (NB) represent only locally infested tissues.

but again we measured no effect of EHS infestation (Table 2, Figure 5D).

Discussion

Exotic herbivores often have devastating effects on naïve hosts. Hemlock woolly adelgid, in particular, is causing widespread mortality in eastern hemlock. The timing of the first generation of HWA attack coincides with the period of rapid shoot growth. The two exotic herbivores studied here have markedly different effects on growth and resource uptake and allocation. Our results suggest that even moderate HWA infestation causes shifts in resource allocation during the first generation of attack. Elongate hemlock scale, in contrast, attacks later in the season after new growth has slowed. This likely explains the mild effects of EHS herbivory on resource allocation. In addition, later in the growing season new shoots often retain the vast majority of the C they receive, with less translocation to other tissues than in early summer (Webb, 1977; Smith & Paul, 1988), potentially explaining the lack of any measurable translocation in response to EHS. Thus, the timing of the attack is likely a key to the contrasting impact of these invasive herbivores. We also observed increased resource allocation to old foliage where HWA was actively feeding, as well as increased belowground resource transport in response to both insects. In the course of this 10-week experiment, we did not observe signs of resource depletion in response to either insect.

Impact on growth and biomass allocation

Hemlock woolly adelgid infestation affected plant growth and biomass allocation within 10 weeks of infestation. Considering that infestation levels were moderate for HWA (Preisser et al., 2008) and EHS (Gómez et al., 2015), the rapidity of this effect is notable.

More specifically, HWA reduced branch elongation by 27% and resulted in a smaller percentage of mass allocated to new needles and branches compared with control trees. Simulated herbivory in conifers has been shown to inhibit shoot growth; shoot length in *Pinus* spp. decreased up to 50% in response to complete defoliation (Honkanen et al., 1994), and shoot length in *Salix* spp. decreased in response to feeding by galling herbivores (Craig et al., 1986).

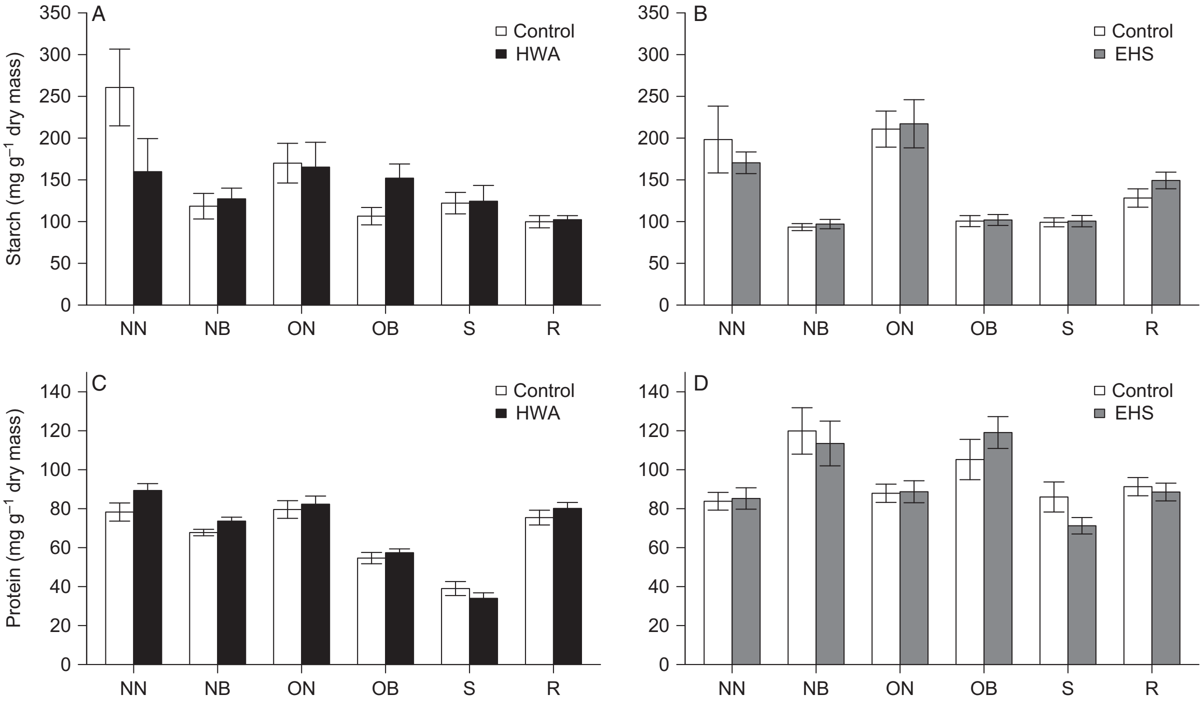

Figure 5 Mean (± SE) starch and protein concentration (mg g^{-1} dry mass) in new needles (NN), new branches (NB), old needles (ON), old branches (OB), main stem (S), and roots (R) of eastern hemlock in response to herbivory by (A, C) hemlock woolly adelgid (HWA) and (B, D) elongate hemlock scale (EHS). In EHS trees, new needles (NN) and new branches (NB) represent only locally infested tissues.

Reductions in new growth not caused by direct consumption likely indicate resource allocation to other tissues and processes, such as storage or defense. Although our results are consistent with the observation that HWA reduces tree growth (McClure, 1991), premature needle abscission (Soltis et al., 2014) could also contribute to the reduction in young needle mass. Stadler et al. (2005) found that needle litterfall is greater beneath infested hemlocks.

In contrast, EHS infestation had no significant effect on growth and only mild effects on biomass allocation. Elongate hemlock scale emerges in June, when the plant has produced most of the season's new growth; this may explain the lack of EHS effect on branch growth. Even so, we did observe a change in biomass distribution: biomass decreased in old needles, and increased in the stem. Elongate hemlock scale widely infested old needles, whereas insect density in new needles was patchier. This suggests old growth is preferred by this insect, and is perhaps one of the reasons why it thrives in adelgid-infested trees which have relatively few young needles. Low biomass in old needles is likely due to premature abscission in these actively infested old needles, a litterfall effect which may contribute to elevated N levels measured beneath infested hemlock stands (Stadler et al., 2005).

Impact on resource pools and allocation

Hemlock woolly adelgid altered translocation patterns of photosynthate, as shown by our ^{13}C pulse-labeling. Notably, HWA increased ^{13}C translocation to the roots and main stem of infested trees compared with control trees. This pattern could imply resource sequestration as a mechanism for induced tolerance: herbivore-induced partitioning of metabolites to inaccessible storage tissues (Orians et al., 2011). Alternatively, it is possible that the increased photoassimilate translocation to roots is a physiological consequence of the reduction in new needle production and subsequent reduction of sink strength in this tissue. In conifers, C allocation shifts toward the roots and latewood stem following the completion of needle elongation in summer (Gordon & Larson, 1968; Smith & Paul, 1988). As summer progresses, the needle-to-stem translocation of assimilates may be localized to starch-storing cells as in *Picea* spp. (Langenfeld-Heyser, 1987), or allocated to the production of thick-walled latewood xylem as in *Pinus* spp., which correlates with current-season needle maturation (Gordon & Larson, 1968). Thus, the increased C translocated to the main stem may be partitioned to starch storage, or to latewood xylem for cell growth. Regardless of the mechanism, this change is associated with reduced growth. We observed a tissue-specific impact of HWA on starch concentration, decreasing starch in new needles, and increasing starch in old branches. Although starch may be partitioned to storage in mature branches, conifers tend to have slightly lower storage than deciduous trees in branches compared with needles (Oren et al., 1988). The slight increase in starch may be related to HWA-induced false ring formation (abnormal cells resembling latewood xylem) in the branches of infested trees (Gonda-King et al., 2012; Domec et al., 2013). This thickening of early- and mid-season cell walls may constitute a stronger C sink in HWA-attacked seedlings, due to the accumulation of C-rich cellulose and lignin in the cell wall.

It is also possible that ^{13}C detected in the stem at the time of sampling may have been en route toward the root. This would act as a compensatory strategy for resource starvation due to herbivory, contributing to the ^{13}C translocation already observed in the root. A previous study of simulated herbivory in *Pinus* indicates increased resource allocation belowground by increasing fine root biomass, rather than sequestration to storage tissues (Moreira et al., 2012).

Although we did not examine herbivory-related water or nutrient stress in this study, long-term infestation may lead to resource starvation. If trees become N-deficient, they can respond by increasing C allocation to fine roots (Dyckmans & Flessa, 2001). In addition, prioritizing carbohydrate storage, as indicated by the increased starch in infested old branches of HWA-infested trees, can aid in maintaining labile N pools and in producing root exudates to improve nutrient cycling (Sala et al., 2012). However, we note that infestation did not cause an increase in N uptake in our study. Thus, further studies of root activity, to differentiate large and fine root growth, and measure root exudation and mycorrhizal colonization, would help to clarify the implications of the observed photosynthate export toward roots.

After one generation of HWA attack, there was a remarkable decrease in starch in new needles as well. A decrease in starch in new needles may indicate resource starvation and decrease long-term growth potential (Sala et al., 2012). In contrast, herbivory effects on N-based pools were weak, with a marginally significant increase in protein within new tissues (see also Gómez et al., 2012). An increase in N pools may increase plant susceptibility to subsequent generations of HWA attack, due to increased palatability and tissue quality (McClure, 1992). We saw no changes to total C or N allocation in response to either insect. Resource pools and storage already accumulated in these seedlings may buffer most bulk C and N effects of our 10-week infestation season.

Nitrogen dynamics were also altered by infestation. The effects of HWA and EHS on ^{15}N translocation were tissue-specific. Hemlock woolly adelgid-infested trees showed increased translocation of ^{15}N to old needles and old branches, and a decrease to new needles and main stem. This indicates that HWA feeding may create a sink for N in the actively infested tissues (old needles and branches) to support insect development. Recent findings of Rubino et al. (2015) in older saplings with long infestation histories, however, suggest that HWA infestation may eventually increase ^{15}N translocation to both old and new needles. Future time-course studies may clarify the link between resource allocation patterns of the first generation and advanced infestation. Whole-plant N uptake was not affected by HWA as hypothesized. Rather our results suggest that the high amino acid composition previously observed in locally infested tissues (Gómez et al., 2012) may be due to changes in N distribution favoring allocation to the infested old branches. Given that protein concentrations did not change, our results do not indicate that HWA can accelerate protein breakdown in the first season of infestation. In EHS-infested trees, N translocation to roots and stems increased. This is consistent with effects of defoliation herbivory on oak seedlings, which increases N allocation to taproots and stem, suggesting induced tolerance through increased storage (Frost & Hunter, 2008). Hemlock woolly adelgid avoids EHS-infested tissue (Gómez et al., 2014) and may be particularly sensitive to N availability (Pontius et al., 2006), and our results provide further support to the idea that changing patterns of nutrition might explain these observations.

Acknowledgements

We are grateful to D Marshall for consultation on statistical methods development, M Rogrove for assistance in the inoculation, care, and harvest of seedlings, S Zollman and L Inouye for the harvest and weighing of tissues for analysis, and two anonymous reviewers for their comments on previous versions of this article. S Gómez was supported by NIFA 2011-67013-30142, L Gonda-King was supported by RI-AES Hatch Grant RH-4001, and N Soltis received funding from the Tufts Institute of the Environment Graduate Student Research Fellowship.

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