

Asymmetric priority effects influence the success of invasive forest insects

MAILEA R. MILLER-PIERCE^{1,2} and EVAN L. PREISSER¹ ¹Department of Biological Sciences, University of Rhode Island, Kingston, Rhode Island, U.S.A. and ²Department of Environmental and Natural Resource Sciences, School of Biological Sciences, Washington State University, Pullman, Washington, U.S.A.

Abstract. 1. Settlement timing is often an important factor in interspecific herbivore interactions, as early-arriving species may encounter higher resource availability and/or avoid induced defences. Despite the general importance of priority effects to the outcome of herbivore interactions, there has been little exploration of such interactions on woody host plants where their impact can only be measured over multiple years.

2. In the eastern U.S.A., two invasive species, the hemlock woolly adelgid *Adelges tsugae* and the elongate hemlock scale *Fiorinia externa*, share a native host, eastern hemlock *Tsuga canadensis*. Their interaction and its consequences were investigated for plant growth – hemlock saplings that had been inoculated with either *A. tsugae* or *F. externa*, starting in spring 2007, were cross-infested with the other insect in spring 2009. A set of uninfested trees was simultaneously infested with *A. tsugae*, *F. externa*, both, or neither insect (= control), and insect density and plant growth was assessed in all treatments.

3. *Adelges tsugae* settlement rates did not differ if it settled alone or simultaneously with *F. externa*, but were ~45% lower on trees previously infested with *F. externa*. There was no difference in *F. externa* settlement rates, and plant growth did not differ substantively between any of the herbivore treatments.

4. At a temporal scale (i.e. multiple growing seasons) appropriate to interactions between woody plants and their herbivores, this work demonstrates that plant-mediated priority effects can substantially affect herbivore settlement and thus the outcome of interspecific competition.

Key words. *Adelges tsugae*, *Fiorinia externa*, herbivory, priority effects, *Tsuga canadensis*.

Introduction

Invasive herbivores often reach high densities in introduced ranges by ‘escaping’ from co-evolved plant defensive compounds as well as predators. The high densities reached by invasive herbivores often mean that when multiple species invade a system, their interactions can have strong community-level effects. Positive feedback between multiple invaders can facilitate subsequent introductions, for example, while negative feedbacks can inhibit later settlement (Simberloff & Von Holle, 1999; Parker *et al.*, 2006). When herbivores differ in their temporal patterns of resource use, the identity of the initially

colonising herbivore can be an important factor in determining host-plant use by later herbivores and can also affect the growth and survival of subsequently colonising species (van Zandt & Agrawal, 2004).

It has been well documented that herbivorous insects can influence each other’s performance via host-plant effects (e.g. induced changes in plant chemistry). The importance of such induced changes in herbivore interactions have been extensively studied (Denno *et al.*, 2000; Fournier *et al.*, 2006). When herbivores share a host plant, alterations of plant chemistry and/or resource levels can affect other species immediately and/or in subsequent generations (Kaplan & Denno, 2007). As a result, the timing of herbivore emergence and feeding is an important factor in competition as earlier emergence may allow an insect to gain a competitive advantage by reaching a nutrient-rich host first. Insects that can avoid

Correspondence: Evan L. Preisser, Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, U.S.A. E-mail: preisser@uri.edu

reductions in host quality (via induced defences and/or reduced host resources) caused by an earlier colonising species (Denno *et al.*, 2000) may also have higher survival. If herbivory reduces host quantity, quality, or increases defences, subsequent colonisers may experience decreased settlement, growth, and fecundity. Cases in which the outcome of species interactions is affected by the order of the species' use of a common resource are commonly referred to as 'priority effects' (Shulman *et al.*, 1983). Understanding the role of priority effects in interactions between sap-feeding insects on woody plants is particularly important; a recent meta-analysis revealed that the impacts of this feeding guild on plant growth and fitness exceed those of defoliating insects (Zvereva *et al.*, 2010). Despite the ecological importance of woody plants, research addressing priority effects in herbivorous species has focused almost exclusively on non-woody plant hosts (e.g. Viswanathan *et al.*, 2007; Erb *et al.*, 2011; however, see Haukioja, 1991).

In the eastern U.S.A., the eastern hemlock *Tsuga canadensis* is a host for two specialist hemipterans from Asia. The first insect, the hemlock woolly adelgid, *Adelges tsugae* Annand is a sessile bivoltine herbivore whose two generations emerge in the early spring and mid-summer. The juvenile 'crawlers' can move actively or be dispersed passively before settling at the base of hemlock needles to feed on xylem parenchyma cells (Young *et al.*, 1995). Adelgid species with a similar life cycle generally inflict minimal damage on their native conifer hosts (Rohfritsch, 1990), and *A. tsugae* populations cause no significant damage in their native range of Japan, Taiwan, mainland China, and western North America (Havill *et al.*, 2006). In the eastern U.S.A., however, they reduce native hemlock growth and can quickly kill even mature hemlocks (McClure, 1991a). The second species, the elongate hemlock scale *Fiorinia externa* Ferris is an armoured diaspidid scale. In New England this insect has one generation per year and, although it has occasionally been found on a number of other native and naturalised tree species, is found almost exclusively on eastern hemlock in this region (McClure & Fergione, 1977). Overwintering adults lay eggs that hatch in late spring; the crawlers settle and become sessile adults that suck fluids from the mesophyll of hemlock needles (McClure, 2002). High-density *F. externa* infestations can lead to reductions in plant growth and overall tree health (McClure, 1980a; Miller-Pierce *et al.*, 2010).

Although both species inhabit the same host in the U.S.A., they differ in some aspects of their ecology. *Adelges tsugae* crawlers emerge in the spring, settle, and begin feeding about 1 month before the emergence of *F. externa* crawlers. *Adelges tsugae* also has one more generation a year than *F. externa* in New England. These differences led to the prediction that *A. tsugae* would exclude *F. externa* in the landscape (McClure, 1997). The recent and rapid range expansion of *F. externa* into *A. tsugae*-dominated hemlock forests, however, suggests that this has not occurred (Preisser *et al.*, 2008b). New England genotypes of *F. externa* were found to have a substantial degree of tolerance to cold overwintering conditions (Preisser *et al.*, 2008a), a fact that may provide a competitive advantage over the relatively cold-sensitive *A. tsugae*. In the only assessment of competition between

the two species, Preisser and Elkinton (2008) conducted a multiyear experimental manipulation that found each species' density was about 30% lower when the species co-occurred than in single species treatments. This study was conducted on individual branches and allowed both species to colonise the resource simultaneously, leaving open the question of whole tree responses and whether priority effects might alter the outcome of the interaction.

We present the results of an experiment addressing the interaction between these two herbivores and the consequences of this interaction for eastern hemlock. Specifically, we conducted a multiyear investigation into the effects of simultaneous versus sequential herbivore colonisation on both species' density and the growth of the shared plant resource. Experimentally manipulating colonisation order allows us to examine whether previous colonisation by one insect had any influence on subsequent colonisation by the other species, and whether this translated into an effect on plant growth.

Methods

We collected eastern hemlock saplings approximately 0.7–1.0 m in height from Petersham (Massachusetts, U.S.A.) in April 2007. This site lay at the northern range boundary for both *A. tsugae* and *F. externa*; careful inspection revealed that neither insect was present on the saplings. To ensure that all of the trees were approximately the same age, we counted visible growth rings on the main stem of 20 similarly sized saplings growing next to the experimental trees; all of the sampled trees were between 9 and 11 years old. Landscape-level surveys confirm that both *A. tsugae* and *F. externa* settle on plants this size in the field (Preisser *et al.*, 2011). Trees were transplanted to a grassy field located within a deer-proof fence at East Farm (Kingston, Rhode Island, U.S.A.), a research facility managed by the University of Rhode Island. The trees were planted within a grid in a randomised complete block design. Treatments were randomly assigned within each row of the grid, and each row contained every treatment. During the summer and autumn of 2007, trees were irrigated when necessary to combat transplant-related water stress; irrigation was discontinued following the 2007 field season. Each sapling was enclosed in a PVC-frame cage covered with mosquito netting (mesh size 97 holes cm⁻²) that allowed ventilation but prevented between-cage crawler dispersal. This netting was also successful at excluding the possibility that the introduced parasitoid *Encarsia citrina* (= *Aspidiotiphagus citrinus*; Hymenoptera, Aphelinidae) might prey upon *F. externa*. Although this parasitoid is rare and inflicts negligible mortality in southern New England (a 2007 survey found that only 3.5% of sampled trees harboured at least one parasitised *F. externa*; Abell, 2010), the adult parasitoids are active in late summer. Despite their potential presence, we never detected any of their distinctive exit holes on adult *F. externa* in our experiment. Cages were removed during the winter to prevent snow-related damage, but were in place before spring crawler emergence. A detailed description of the experimental design and inoculation protocol follows.

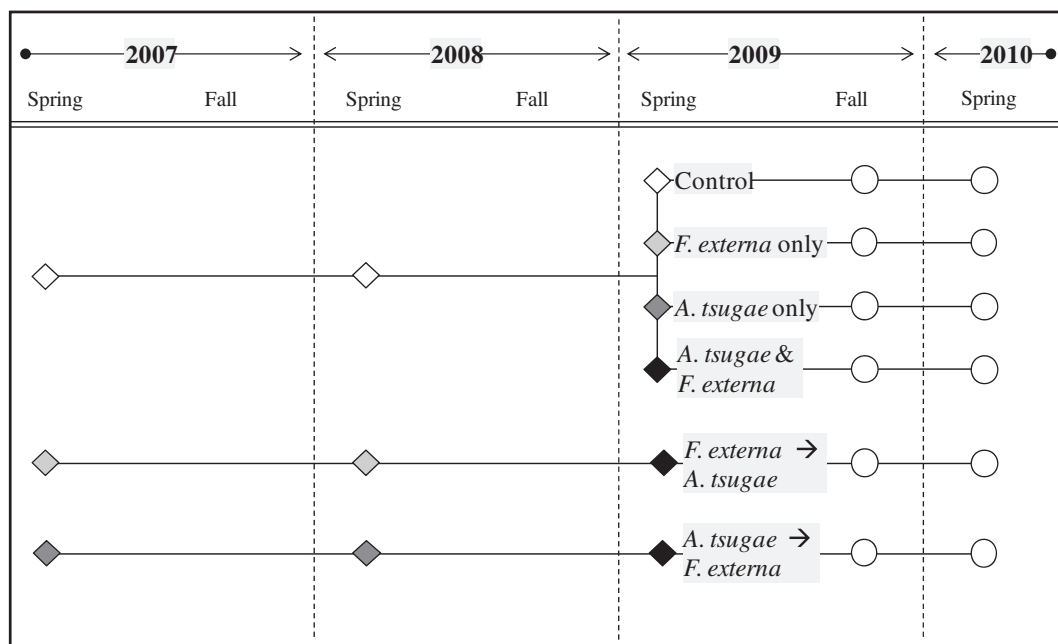


Fig. 1. Design of priority effects experiment. Diamonds indicate insect inoculations (open symbols: no insects; light grey symbols: *Fiorinia externa* only; dark grey symbols: *Adelges tsugae* only; black symbols: both *F. externa* and *A. tsugae*). Open circles indicate sampling dates (October 2009 and March 2010).

Experimental design

Trees in the '*A. tsugae* → *F. externa*' treatment had *A. tsugae* applied in spring 2007, 2008, and 2009 (see below for details and rationale behind this approach); in spring 2009, the trees in this treatment were also inoculated with *F. externa* (Fig. 1). All inoculations were carried out using hemlock foliage infested with gravid adults of the appropriate species, a standard approach in this system (Butin *et al.*, 2007); this procedure was necessary as the crawlers of both species are so small, and their survival rate so low, that working with them directly in the field is impractical. The '*F. externa* → *A. tsugae*' treatment had *F. externa* applied in spring 2007, 2008, and 2009; in spring 2009, the trees in this treatment were also inoculated with *A. tsugae*. Trees in the '*A. tsugae*-only' treatment had uninfested foliage applied in spring 2007 and 2008, but in spring 2009 were inoculated with *A. tsugae*-infested foliage. Trees in the '*F. externa*-only' treatment had uninfested foliage applied in spring 2007 and 2008, but in spring 2009 were inoculated with *F. externa*-infested foliage. Trees in the '*A. tsugae* & *F. externa*' treatment had uninfested foliage applied in spring 2007 and 2008, but in spring 2009 were inoculated with both *A. tsugae*-infested foliage and *F. externa*-infested foliage. Trees in the 'control' treatment had uninfested foliage applied to them through the experiment (spring 2007, 2008, and 2009). Although the experiment initially had a balanced number of experimental replicates, tree mortality and inadvertent cross-contamination over the 4-year course of the experiment reduced the '*A. tsugae*-only', '*F. externa*-only', and '*F. externa* → *A. tsugae*' treatments to eight replicates each (the '*F. externa*-only' treatment declined to seven replicates in

March 2010 because one tree was accidentally damaged during the winter); all other treatments had nine replicates.

Selection of insect populations used for inoculation

Although *A. tsugae* crawlers emerge approximately 1.5 months earlier in the spring than do *F. externa* crawlers (McClure, 1978, 1991a), we could not eliminate the possibility that late-emerging *A. tsugae* crawlers might still be present at the beginning of *F. externa* crawler emergence. As a result, we chose not to use branches infested with both insects for our inoculation protocols. Although this precaution was necessary to avoid cross-contamination, it meant that we had to locate areas within the invaded range where only one of the two species was present (preventing us from collecting both *A. tsugae*- and *F. externa*-only branches from the same location). In 2007, we used branches from a known *A. tsugae*-only population in Amherst (Massachusetts, U.S.A.), and located a *F. externa*-only population in East Granby (Connecticut, U.S.A.). In 2008, a resurvey of both collection localities revealed that the *F. externa*-only site remained free of adelgid but that *F. externa* had invaded the formerly *A. tsugae*-only stand in Amherst. This led us to conduct a survey of *A. tsugae*-infested stands for populations that remained free of *F. externa*. We discovered such a stand in Kingston, and used it as a source of *A. tsugae*-only foliage for the 2008 and 2009 inoculation cycles. Because the *F. externa*-only site in East Granby remained free from *A. tsugae* throughout the experiment, we continued to use it as our source of *F. externa*-only foliage.

Inoculation timing

We chose to replicate the natural patterns of *A. tsugae* and *F. externa* settlement by re-inoculating our saplings on a yearly basis (although *A. tsugae* has two generations per year, the overwintering generation is $\sim 10\times$ more fecund than the rapidly maturing summer generation; McClure, 1989). In 2008 and 2009, this meant that saplings in the '*A. tsugae* \rightarrow *F. externa*' and '*F. externa* \rightarrow *A. tsugae*' treatments could be infested by both externally produced crawlers (i.e. from the inoculant branches) and locally produced crawlers (i.e. from successfully overwintering adults on the sapling itself). Our March 2008 and 2009 surveys found overwintering adults in both the '*A. tsugae* \rightarrow *F. externa*' and '*F. externa* \rightarrow *A. tsugae*' treatments (Appendix I).

Inoculation protocol

Because *A. tsugae* crawlers emerge earlier in the spring than *F. externa* crawlers (McClure, 1978, 1991a), our inoculation protocol consisted of two stages. Immediately following the first signs of local *A. tsugae* crawler emergence, we collected *A. tsugae*-infested foliage from Amherst (in 2007) or Kingston (in 2008 and 2009) and uninfested foliage from Exeter (Rhode Island, U.S.A.). In early April 2007 and 2008, we first applied *A. tsugae*-infested foliage to each sapling in the '*A. tsugae* \rightarrow *F. externa*' treatment. The following day, we applied uninfested foliage to saplings in each of the other treatments. This application of uninfested foliage was done to ensure that all treatments experienced identical disturbance regimes. We ensured crawler transfer onto the experimental saplings by employing inoculation methods proven to produce high rates of crawler settlement (Butin *et al.*, 2007). In late May 2007 and 2008, we collected *F. externa*-infested foliage from East Granby and uninfested foliage from Exeter. After removing the desiccated foliage from the first inoculation (all emerging *A. tsugae* crawlers having either settled or died), we applied *F. externa*-infested foliage to each sapling in the '*F. externa* \rightarrow *A. tsugae*' treatment. The following day, we applied uninfested foliage to saplings in each of the other treatments. After 2 months, we returned and removed the desiccated foliage from each sapling.

In 2009, we changed the inoculation protocol as follows (Fig. 1). First, in addition to using *A. tsugae*-infested foliage to inoculate saplings in the '*A. tsugae* \rightarrow *F. externa*' treatment, we also used it to inoculate saplings in the '*A. tsugae*-only', '*F. externa* \rightarrow *A. tsugae*', and '*A. tsugae* & *F. externa*' treatments. Second, in addition to using *F. externa*-infested foliage to inoculate saplings in the '*F. externa* \rightarrow *A. tsugae*' treatment, we also used it to inoculate saplings in the '*F. externa*-only', '*A. tsugae* \rightarrow *F. externa*', and '*A. tsugae* & *F. externa*' treatments. Other than these two changes, the 2009 protocol was identical to 2007/2008.

Data collection

We censused insect populations twice per year in 2007–2010. During each census, we counted *A. tsugae* and

F. externa densities on 12 randomly selected branchlets per tree (three branchlets on each of four branches). We recorded the number of *A. tsugae* present per cm growth on the current year's foliage ('newest growth'). We counted *F. externa* on 50 needles of new growth; in high densities, we counted the number of needles necessary to find 25 individuals. The number of *F. externa* per needle was converted to *F. externa* per cm foliage by multiplying the former value by $10.32 \text{ needles cm}^{-1}$ (see Miller-Pierce *et al.*, 2010 for the source of this conversion). Counts were conducted using a magnifying visor to assist in detecting aestivating *A. tsugae* nymphs that possess only a small amount of wool and are generally not visible to the naked eye (note: this life stage was only present during the October sampling dates).

The first sampling date, October of 2007/2008/2009, assessed insect density once *A. tsugae* and *F. externa* populations had settled and begun to mature (but see the previous paragraph regarding aestivating *A. tsugae* nymphs). The second sampling date, March of 2008/2009/2010, took place before emergence of either species' crawlers and assessed density as well as overwintering survival of the insects counted in October of the previous year. While a third mid-summer sampling date would have been ideal, early-instar *F. externa* crawlers hide under the opaque white midrib of hemlock needles and are virtually impossible to find and count without the aid of a dissecting microscope. In October 2009, we also assessed plant growth by measuring new growth (cm) on the 12 branchlets per tree that were used for density measurements. Because new growth is produced from May to July/August, plant growth measurements were not taken in March 2010. Data on insect densities in the '*A. tsugae* \rightarrow *F. externa*' and '*F. externa* \rightarrow *A. tsugae*' treatments in 2007, 2008, and March 2009 are contained in Appendix I. Although we sampled all of the other treatments at the same time, neither *A. tsugae* nor *F. externa* were detected on any of the other saplings.

Statistical analysis

All analyses were conducted using JMP 9.0 (SAS, 2010) with each data point being the mean response per tree per sampling date. For our analyses of the autumn 2009 and spring 2010 data on insect density, the main effects of treatment, time, column (used as a blocking variable to account for grid location bias), and the treatment \times time interaction were tested using repeated measures ANOVA. For our analyses of the autumn 2009 data on plant growth, the main effects of treatment and column were tested using one-way ANOVA. If the factor 'column' was non-significant, it was removed and the test repeated. All data were checked for normality and homogeneity of variances; data on *F. externa* density was square-root transformed to meet the assumptions of ANOVA. We used Student's *t*-test ($\alpha = 0.05$) to conduct means separation tests when necessary. When initial *P*-values are significant, we report both the initial *P*-value as well as the *P*-value corrected for multiple comparisons within each analysis at $\alpha = 0.05$ using step-up false discovery rate, a sequential Bonferroni-type procedure (Benjamini & Hochberg, 1995).

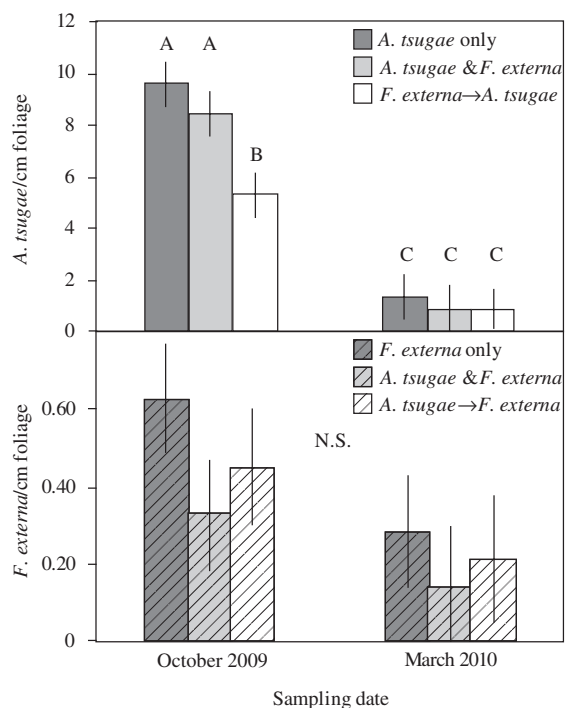


Fig. 2. Insect density in October 2009 and March 2010. (a) *Adelges tsugae* settlement on trees first infested with *A. tsugae* in 2009 (dark grey bars), trees simultaneously infested with *A. tsugae* and *F. externa* in 2009 (light grey bars), and trees infested with *Fiorinia externa* since 2007 (white bars). (b) *Fiorinia externa* settlement on trees first infested with *F. externa* in 2009 (dark grey hatched bars), trees simultaneously infested with *A. tsugae* and *F. externa* in 2009 (light grey hatched bars), and trees infested with *A. tsugae* since 2007 (white hatched bars). Bars indicate least square means \pm standard error and letters indicate significant differences between groups (Student's *t*-test with $\alpha = 0.05$). N.S., no significant between-treatment differences.

Results

Adelges tsugae density

After the spring 2009 inoculations, *A. tsugae* settlement in autumn 2009 differed among treatments ($F_{2,22} = 3.90$, $P_{\text{initial}} = 0.036$; $P_{\text{adjusted}} < 0.05$) (Fig. 2a). There was no difference between the '*A. tsugae*-only' and '*A. tsugae* & *F. externa*' (simultaneous colonisation) treatments; however, *A. tsugae* densities were 44% lower when *A. tsugae* settled on branches already colonised by *F. externa* (the '*F. externa* → *A. tsugae*' treatment) versus when settling alone (Student's *t*-test, $P < 0.05$). There was a significant effect of time, a consequence of overwintering mortality between October 2009 and March 2010 ($F_{1,22} = 117.28$, $P_{\text{initial}} < 0.0001$; $P_{\text{adjusted}} < 0.05$). There was also a significant time \times treatment interaction ($F_{2,22} = 4.24$, $P_{\text{initial}} = 0.028$; $P_{\text{adjusted}} < 0.05$); although *A. tsugae* densities in March 2010 were still 35% lower in the '*F. externa* → *A. tsugae*' treatment than in the '*A. tsugae*-only' treatment, the between-treatment differences were no longer significant (Student's *t*-test, $P > 0.05$).

Fiorinia externa density

Densities of *F. externa* were substantially lower than those of *A. tsugae* in all treatments, probably due to several days of abnormally cold weather immediately following the application of the *F. externa* inoculants (Fig. 2b). Despite the overall lower densities, there were no among-treatment differences in *F. externa* settlement rates during the experiment ($F_{2,21} = 1.27$, $P_{\text{initial}} = 0.30$). Overwintering mortality led to a decline in *F. externa* densities between October 2009 and March 2010 ($F_{1,21} = 6.16$, $P_{\text{initial}} = 0.022$; $P_{\text{adjusted}} < 0.05$), but there was no time \times treatment interaction ($F_{2,21} = 0.04$, $P = 0.96$).

Plant growth

New growth differed significantly among treatments ($F_{5,45} = 11.4$, $P_{\text{initial}} < 0.001$; $P_{\text{adjusted}} < 0.05$) (Fig. 3). New growth in the control was significantly higher than in any of the herbivore addition treatments (Student's *t*-test, $P < 0.05$). Within the herbivore treatments, the '*F. externa*-only' treatment had greater growth than the '*A. tsugae* & *F. externa*' treatment, but there were no other significant among-treatment differences (Student's *t*-test with $\alpha = 0.05$).

Discussion

Initial resource conditions can be an important determinant of interspecific herbivore competition (Kaplan & Denno, 2007; Anderson *et al.*, 2009). Our results show that previous, but not simultaneous, colonisation by one invasive hemipteran impacts subsequent colonisation by a second species in the same guild. *Adelges tsugae* settlement densities were significantly lower on trees with *F. externa* present for 2 years than when *A. tsugae* settled alone (Fig. 2a). While it may seem unsurprising that two previous years of *F. externa* herbivory might alter host quality sufficiently to affect *A. tsugae*, the opposite result did not happen: *F. externa* settlement was not significantly affected by 2 years of previous colonisation of the host by *A. tsugae* (Fig. 2b). Although *A. tsugae* densities in the '*F. externa* → *A. tsugae*' treatment were still 35% lower than in

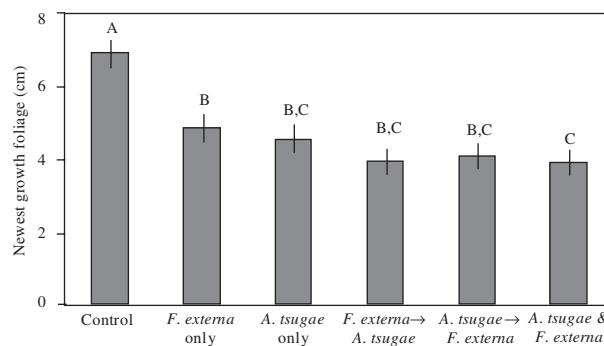


Fig. 3. Length of newest growth foliage (cm) in October 2009. Bars indicate least square means \pm standard error and letters indicate significant differences between groups (Student's *t*-test with $\alpha = 0.05$).

the 'A. tsugae-only' treatment in March 2010, this difference was no longer statistically significant (likely because of our relatively low replication). It is important to note, however, that even though equal numbers of overwintered adult insects were also applied to trees in both treatments in March 2009 to start the priority effects experiment, there was none the less a substantial difference in subsequent adelgid density. As peak adelgid densities (especially in the spring-emerging generation; McClure, 1991a) are primarily responsible for hemlock mortality, however, a decrease in summer/autumn adelgid counts could still prove beneficial over a multiyear period.

In light of the long-term and large-scale nature of our experiment, findings suggest a largely asymmetric interaction in which *F. externa* makes the tree less suitable for *A. tsugae* settlement through exploitative resource competition and/or the induction of plant defences. In other words, herbivore-induced priority effects decreased the success of *A. tsugae* but not *F. externa*. Other alternative explanations such as interference competition are unlikely as *A. tsugae* and *F. externa* crawlers do not overlap temporally and the adults are sessile. Apparent competition can also be ruled out because neither insect appears to possess natural enemies capable of limiting their population growth in the invaded range (Danoff-Burg & Bird, 2002). Even if effective natural enemies were present, however, the mesh cage around each tree appears to have sharply limited predator/parasitoid access during the periods of greatest insect abundance (i.e. early spring through late autumn; see Methods for details).

Exploitative competition between *A. tsugae* and *F. externa* is the most likely explanation for our results. Although a recent review of interspecific herbivore interactions concluded that resource quantity *per se* is often a relatively unimportant factor in competition (Kaplan & Denno, 2007), resource quality may play a key role in this system. *Adelges tsugae* requires high-quality hemlock foliage; crawlers settling on anything other than newly produced branches have extremely low survival rates (McClure, 1991a), and nitrogen addition increases *A. tsugae* survival and fecundity (McClure, 1991b). *Adelges tsugae*-infested foliage has also been found to contain higher nitrogen content than uninfested foliage (Stadler *et al.*, 2006), and a recent experimental assessment found a 330% increase in total amino acid concentration in *A. tsugae*-infested hemlock foliage versus uninfested foliage after 1 year (Gómez *et al.*, 2012). In addition, hemlocks infested with *A. tsugae* reallocate resources for growth towards new growth foliage, leading to a large decrease in the size of old growth needles (Radville *et al.*, 2011). These findings suggest that *A. tsugae*, like other adelgids, may be capable of manipulating host physiology to form resource-rich 'islands' at the cost of overall plant health (Havill & Footitt, 2007). In contrast, *F. externa* decreased total foliar amino acid concentration by ~25% relative to uninfested hemlock foliage 1 year after inoculation (Gómez *et al.*, 2012). Although *F. externa* benefits from increased foliar nitrogen, it can survive and reproduce on poor-quality foliage (McClure, 1979, 1980b) and is often extremely abundant on stressed hemlocks (E. Preisser, unpublished). Their increased abundance on stressed trees may be an

example of the plant stress hypothesis, which states that host plants that reduce defence allocation and increase amino acid concentrations in response to physiological challenges may inadvertently improve their palatability to sessile herbivores (e.g. Mattson & Haack, 1987).

The relatively innocuous effects of *F. externa* on hemlock health (Miller-Pierce *et al.*, 2010; Gonda-King *et al.*, 2012) may explain the absence of any significant competitive interaction on insect densities in the simultaneous 'A. tsugae & F. externa' colonisation treatment. As *F. externa* establishes and begins to reproduce, however, its density and overall impact on foliar quality increases (McClure, 1980b) and the host becomes less suitable for *A. tsugae* survival and reproduction (i.e. the plant vigour hypothesis; Price, 1991). The asymmetry of this interaction may thus be explained by the relative tolerance of *F. externa* to poor host conditions (McClure, 1980b). If so, the *A. tsugae*-*F. externa* interaction may parallel the one occurring between the pine scale *Matsucoccus resinosa* and the pine adelgid *Pineus boernerii* (McClure, 1990). The scale excludes the adelgid from feeding on their shared host, the red pine *Pinus resinosa*. Although both species cause similar damage to their host, the adelgid requires high-quality plant material to reproduce while the scale is capable of both reproducing and persisting at low resource levels, and is able to competitively exclude *P. boernerii* over time (McClure, 1990). A similar interaction has been documented between two sap-feeding planthoppers, one of which, *Prokelisia dolus*, is more tolerant of low N levels than *P. marginata*. The superior competitive ability of *P. dolus* was attributed to its capacity to compensate for low nutrients by increasing phloem sap intake (Denno *et al.*, 2000). Although we are unaware of any research assessing the nutritional requirements of *F. externa* and *A. tsugae*, it seems likely that these two species differ in this regard.

It is also possible that the asymmetric interaction between *A. tsugae* and *F. externa* is the result of plant defence induction. Although long-lived woody plants have been hypothesised to rely more on constitutive versus induced defences (Karban & Baldwin, 1997), there are examples to the contrary (Nykänen & Koricheva, 2004). Interspecific herbivore competition often hinges on changes in plant quality (Kaplan & Denno, 2007), and researchers have documented defence-mediated priority effects in a number of systems (reviewed in Erb *et al.*, 2011). Viswanathan *et al.* (2005), for example, found that previous feeding by the beetle *Psylliodes affinis* induced plant responses and reduced host quality in *Solanum dulcamara*, making it a less suitable resource for the later-arriving beetle *Plagiometriona clavata*. These induced responses persisted through time and decreased *P. clavata* densities in subsequent generations. Although temporal variation can complicate predictions of the outcome(s) of multiple herbivore-plant interactions (van Zandt & Agrawal, 2004), herbivores are generally consistent in their ability to elicit specific host responses. Evidence that plant defences may be involved in this system comes from research evaluating whether feeding induces a hypersensitive response (assessed using hydrogen peroxide levels in foliage; Radville *et al.*, 2011). While both *A. tsugae* and *F. externa* induced

a localised hypersensitive response, *A. tsugae* also induced systemic hypersensitivity: H₂O₂ levels in newly produced uninfested foliage adjacent to older *A. tsugae*-infested foliage were only slightly lower than in the older infested foliage. In contrast, *F. externa* did not increase H₂O₂ levels in uninfested foliage (Radville *et al.*, 2011). This result implies that *A. tsugae* should, if anything, have a greater inhibitory effect on *F. externa* than vice versa. This prediction runs contrary to our findings, suggesting that plant hypersensitivity is not the driving force behind the suppression of *A. tsugae* by *F. externa*.

Regardless of the mechanistic basis of the *A. tsugae*–*F. externa* interaction, our results provide additional evidence that *F. externa* may be capable of inhibiting *A. tsugae* populations. The ability of *A. tsugae* to rapidly colonise and infest healthy hemlock trees led to the prediction that it would eventually exclude *F. externa* from the New England landscape (McClure, 1997). In reality, however, landscape-level surveys documenting the range expansion of *A. tsugae* and *F. externa* throughout New England found that *F. externa* populations had increased and *A. tsugae* populations had decreased between 1997 and 2005 (Preisser *et al.*, 2008b). The only other experimental work addressing the *A. tsugae*–*F. externa* interaction, a multiyear study in which both insects simultaneously colonised individual hemlock branches, found that each species reduced the other's density by approximately 30% (Preisser & Elkinton, 2008). Although such symmetrical effects may seem to conflict with the results reported here, the reductions occurred over a longer time frame than this work and were not manifest until 1.5 years after the initial inoculations. At the landscape level, initial fears that *A. tsugae* would extirpate hemlocks from southern New England (Orwig & Foster, 1998) have not been realised and stand-level mortality in this area is occurring much more slowly than predicted (Preisser *et al.*, 2008b). Although overwintering mortality of *A. tsugae* due to cold weather undoubtedly plays a role in reducing hemlock mortality (Parker *et al.*, 2002), our results suggest that the rapid northward range expansion of *F. externa* into *A. tsugae*-invaded forests, whether driven by increased *F. externa* cold tolerance (Preisser *et al.*, 2008a) or some other factor(s), may also impact stand health.

Although the potential for *F. externa*-mediated reduction of *A. tsugae* densities may appear a promising result from a management perspective, there are several notes of caution. First, hemlock growth in all treatments was significantly lower than in the control after 6 months (Fig. 3). This result conflicts with other long-term work, which found that *A. tsugae* had a much greater effect on plant health than *F. externa* (Miller-Pierce *et al.*, 2010). Previous experimental work on *A. tsugae*–*F. externa* interactions observed the same immediate reduction in plant growth (fig. 2a in Preisser & Elkinton, 2008), however, and found that differences between insect treatments took several years to manifest. Second, the hemlocks in this experiment grew in an open field (necessary because of the need for a deer-fenced area adjacent to a water source for irrigating the newly transplanted hemlocks during the 2007 field season) in which abiotic conditions

were very different from the heavily shaded forest interiors in which hemlock often grows. Third, we conducted our research with saplings because we could not cage and experimentally manipulate mature trees (i.e. >10 m in height). While this may have affected our results, both *A. tsugae* and *F. externa* readily colonise saplings as well as mature trees in the field (Preisser *et al.*, 2011), and *A. tsugae*-infested saplings and mature trees do not differ in their defensive chemistry or degree of terpene induction (J. Elkinton, unpublished). Finally, our results may have been affected by the fact that *A. tsugae* overwintering mortality rates are often high in New England (Parker *et al.*, 2002); experimental work in the warmer southern portion of the invaded range is needed.

While the importance of interspecific competition in dictating the total impact of herbivory has long been recognised, the role(s) of temporal variation and priority effects have received less attention. We document an asymmetric priority effect manifest over several years in which previous settlement by a relatively benign herbivore reduces the densities of a more harmful species. The most likely explanations for this result involve herbivore-induced changes in plant quality and/or defensive chemistry. While the impact of priority effects on herbivore settlement and growth have recently been documented in herbaceous plants (e.g. Viswanathan *et al.*, 2007; Erb *et al.*, 2011), ours appears to be the first work addressing such reciprocal interactions in woody plants at an appropriate temporal scale (i.e. multiple growing seasons). In addition to the potential management implications, our results support the argument (Anderson *et al.*, 2009) that longer-term studies of interspecific herbivore competition may provide very different results than short-term work.

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Appendix I

Densities of EHS (top panel) and HWA (bottom panel) in the EHS→HWA and HWA→EHS treatments, respectively, from October 2007 through March 2009. This data is also presented in Gomez *et al.*, 2012, and is provided here solely to confirm the presence of overwintered adults in each treatment prior to the start of the priority effects manipulation. Data from the October 2009 and March 2010 density counts are contained in Fig. 1 of the main text. All of the other treatments were sampled at the same time, but neither HWA nor EHS were detected on saplings assigned to any of the other treatments. Red arrows indicate timing of yearly insect inoculations following the March density counts. The extremely high EHS densities in March 2009 may be due to experimenter

error; we censused trees for EHS during a late-winter snowstorm (<http://newsblog.projo.com/2009/03/march-sends-a-r.html>), but waited several days for the snow to melt before doing HWA counts. This delay was necessary because the waxy white coating that characterizes mature HWA is essentially indistinguishable from snow.

