Agricultural and Forest Entomology

Agricultural and Forest Entomology (2020), DOI: 10.1111/afe.12382

Differential sunlight exposure affects settling behaviour of hemlock woolly adelgid crawlers

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Abstract

1 Previous research demonstrated that elevated sunlight improves carbon balance and growth of eastern hemlock (*Tsuga canadensis* (L.) Carrière) seedlings infested with the invasive hemlock woolly adelgid (*Adelges tsugae* Annand).

2 This study examined the hypothesis that elevated visible and ultraviolet sunlight directly affects the settling behaviour of *A. tsugae* crawlers.

3 Hemlock seedlings were manually infested with equal densities of *A. tsugae* and placed into artificial shade treatments (0%, 40% and 80% shade). The density of settled nymphs of the ensuing generation on shaded seedlings was three times higher than the density on unshaded seedlings.

4 *Adelges tsugae* ovisacs were also attached to hydrated hemlock branches placed beneath acrylic filter treatments that selectively allowed transmission of various sunlight wavelengths. The proportion of adelgid crawlers that settled on the branch (*vs.* dropping from it) was higher beneath filters that blocked the widest spectrum of solar radiation (opaque and amber filters) than beneath full sun. In the March-April trial, exposure to full sun and the full visible spectrum only (clear-UV filter) significantly increased the proportion of crawlers that settled on the bottom (*vs.* top) side of the branch.

5 Our results suggest that *A. tsugae* crawlers are negatively phototactic and/or thermotactic, with behaviour influenced more by visible light than ultraviolet light.

Keywords *Adelges tsugae*, eastern hemlock, forest insects, invasive species, negative phototaxis, shade, *Tsuga canadensis*, ultraviolet light, visible light.

Introduction

The hemlock woolly adelgid *Adelges tsugae* Annand (Hemiptera: Adelgidae) is a non-native invasive forest insect in eastern North America. It has caused widespread decline and mortality of eastern hemlock *Tsuga canadensis* (L.) Carrière, a long-lived, shade-tolerant, evergreen conifer that functions as a 'foundation species' in stands where it occurs as a canopy dominant or codominant (Ellison *et al.*, 2005). Forest stand changes associated with *A. tsugae* infestation include altered carbon, nutrient and hydrologic cycling, altered microclimates and a shift toward dominance of non-hemlock vegetation (Orwig *et al.*, 2008; Ford *et al.*, 2012; Dharmadi *et al.*, 2019). Substantial economic and aesthetic impacts are associated with the decline and loss of large eastern hemlocks in residential and recreational areas (Holmes *et al.*, 2005; Aukema *et al.*, 2011).

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Hemlock woolly adelgid is also a pest of Carolina hemlock *Tsuga caroliniana* Engelmann, which occupies a limited range in the Appalachian Mountains of the southeastern United States (Jetton *et al.*, 2013).

In North America, *A. tsugae* completes two asexual generations per year: the sistens generation occurs from summer through early spring, whereas the progrediens generation occurs from spring through early summer. A proportion of the progrediens generation develops as winged adult females called sexuparae, but this stage does not reproduce in North America due to absence of the primary spruce host (McClure, 1989; Havill *et al.*, 2016). Otherwise, adults of both asexual generations are sessile and cover themselves with a white, waxy secretion that forms a globular ovisac within which they lay their eggs. Wingless first-instar nymphs, known as crawlers, disperse from these ovisacs in search of feeding sites at the base of hemlock needles. Crawlers may ultimately settle on their natal plant, fall to the ground or be passively dispersed by wind and animals such as birds (McClure, 1990; Russo *et al.*, 2019). Crawlers of the progrediens generation disperse in March and April and settle on previous-year hemlock branchlets (where their mothers also settled), whereas sistens crawlers disperse in May and June and settle on current-year branchlets.

A number of recent studies suggest that elevated levels of natural sunlight improve the vigor of eastern hemlock trees in the presence of *A. tsugae*. Cumulatively, these studies indicated that elevated sunlight exposure is associated with (i) lower densities of adelgid on hemlock foliage (Hickin & Preisser, 2015; Brantley *et al.*, 2017, McAvoy *et al.*, 2017) and (ii) improved hemlock carbon gain and superior growth during infestation (Brantley *et al.*, 2017; Miniat *et al.*, 2020). The mechanisms contributing to the negative association between elevated sunlight and *A. tsugae* densities are uncertain, but may include (i) direct effects of sunlight on insect behaviour or survival (Shimoda & Honda, 2013; Hori *et al.*, 2014), (ii) indirect effects mediated through physiological changes in the host plant or (iii) both (Lapham *et al.*, 2018).

In this study, we conducted two experiments to further evaluate the hypothesis that increased sunlight exposure reduces *A. tsugae* density on eastern hemlock. In the first experiment, we applied varying levels of artificial shade to seedlings infested with equal densities of *A. tsugae*, and examined the effect on adelgid densities in subsequent generations. In the second experiment, we examined the hypothesis that sunlight directly affects crawler settling behaviour by examining the effect of various sunlight filters on the proportion of crawlers settled on cut hemlock branches, and their location (top *vs*. bottom side of the branch).

Materials and methods

Shade cloth experiment

An experiment was conducted to investigate the effect of various sunlight intensity levels on *A. tsugae* densities on potted *T. canadensis* seedlings pre-infested with equal numbers of adelgids. In January 2012, 120 bare root 4-year-old *T. canadensis* seedlings (2-2 transplants) were obtained from Pikes Peak Nursery (Penn Run, Pennsylvania). Each seedling was potted in a 6 L plastic pot (Steuwe & Sons, Tangent, Oregon) using a commercial soil medium (Fafard® 3B growing mix, Sun Gro Horticulture, Agawam, Massachusetts), fertilized with 15 g of Multicoat® 15-16-17 granular slow-release fertilizer (Haifa Group, Altamont Springs, Florida) and hydrated with tap water. Seedlings were watered regularly and maintained in a greenhouse at the North Carolina Department of Agriculture and Consumer Services/North Carolina State University Mountain Research Station near Waynesville, NC (35.4876° N, 82.9670° W) for 2 months to allow seedlings to become established in the pots.

Seedlings were artificially infested with *A. tsugae* crawlers of the progrediens generation in March 2012. Infested *T. canadensis* branch material was field-collected in McDowell County, NC (35.6068° N, 82.1004° W) and Morgan County, TN (36.0940° N, 84.4091° W) and microscope examination of 15, 20-cm twigs from this material revealed a mean (SE) of 383 (32) sistens ovisacs per twig and 41 (2) progrediens eggs per ovisac. One 20-cm *T. canadensis* twig of this same material was loosely attached to each seedling with a wire twist-tie, delivering ~15 700 eggs per seedling. Seedlings were watered regularly and housed in a concrete block building (sheltered from rain, wind and direct sunlight) for two months to allow progrediens crawlers to hatch, disperse and settle before placement of the seedlings into shade treatments.

In May 2012, the density of *A. tsugae* progrediens ovisacs on each seedling was estimated nondestructively by subsampling five 10 cm branchlets per seedling (the terminal shoot and 4 lateral branchlets). Seedlings were then divided into 15 eight-seedling groups such that the mean density of progrediens adults per shoot was similar in each group (~45 adults per 10 cm shoot). Each group of eight seedlings was then randomly assigned to one of 15 shade treatment plots, arranged in five blocks with three shade treatments (0%, 40% and 80% shade) per block. Treatment plots were located in an open field and the ground surface was covered with black polypropylene ground fabric (Greenhouse Megastore, Danville, Illinois) to prevent weed growth. Treatment plots were 1.8 m × 1.8 m square with 1 m vertical metal T-posts inserted into the ground at each corner, and a 1.2 m T-post inserted in the plot centre. For the 40% and 80% shade treatments, posts were covered to create a roof and sides of knitted polyethylene black shade cloth (Greenhouse Megastore) rated to provide the target level of shade. The top ends of the metal T-posts were covered with PVC end-caps to prevent damage to the shade cloth. In the 0% shade treatment, no cloth was applied and seedlings were in full sun. Seedlings were arranged in a circle around the centre post and cypress mulch was packed around each pot to a depth of 10–15 cm to insulate the roots and soil during the winter. Seedlings were watered regularly (twice weekly for 2–4 h, adjusted as needed to local weather and rainfall conditions) during the spring, summer and fall with a button dripper irrigation system (Drip Depot, Inc., White City, Oregon) supplying 1.9 L water per hour to the soil surface of each pot.

Seedlings were artificially infested with *A. tsugae* progrediens crawlers a second time in March 2013. The infestation method was the same as in March 2012 except that the seedlings were infested in-place within the shade treatment plots. Source material for the second artificial infestation was collected from infested eastern hemlock in Buncombe County, NC (35.6120° N, 82.5622° W). Based on destructive counts of a subsample of 15, 20-cm twigs from this material, the second infestation introduced a mean (SE) of 194 (24) sistens ovisacs and 36 (3) progrediens eggs per ovisac to each seedling (~7000 eggs per seedling).

In addition to the *A. tsugae* density counts on seedlings in May 2012 prior to placement in the shade tents (pre-treatment), adelgid density was assessed post-treatment at four times throughout the experiment. Densities of sistens nymphs in November 2012 and progrediens adults in June 2013 were estimated using nondestructive counts of adelgids on five, 10 cm branchlets per seedling (terminal shoot and 4 lateral branchlets). Nondestructive whole-seedling counts were used to estimate densities of sistens adults in February 2013 and sistens nymphs in November 2013 due to very low densities of adelgids that could not be accurately tallied using 10-cm branchlet samples.

Acrylic filter experiment

March–April trial. A field experiment with two trials was conducted in 2018 to examine the effect of filtering visible and ultraviolet light on *A. tsugae* crawler settling behaviour. Thirty-two uninfested branches, 25 cm long, were clipped from two healthy *T. canadensis* trees growing in full sun near Cleveland, SC (35.0731° N, −82.5989° W). The cut ends of these branches were inserted in floral foam (Oasis Instant Deluxe, Smithers-Oasis Company, Kent, Ohio) saturated with tap water, transported to the laboratory and placed in a refrigerator at 3 °C overnight. Separate branch material infested with *A. tsugae* sistens ovisacs containing progrediens eggs were clipped from *T. canadensis* trees in Asheville, NC (35.6139° N, −82.5637° W). The mean (SE) number of eggs per ovisac on the infested material was 194 (15) based on a sample of 20 dissected ovisacs. This material was then clipped into 5–10 cm long segments each harboring 10 ovisacs, and one infested segment was attached in the centre on the top side of each uninfested 25-cm branch (described above) using a wire twist-tie. Approximately 3 cm of stem from the cut end of the 25-cm branch was inserted into a cylindrical plastic film canister (5 mm long, 30 mm diameter) containing floral foam saturated with tap water and sealed on the open end with laboratory film (Parafilm M, Pechiney Plastic Packaging, Menasha, Wisconsin).

Each of the 25-cm hydrated branches was placed on top of separate white plastic boxes, each measuring 19 × 10 × 7.6 cm (L × W × H) (model S-12414, Uline Shipping Supplies, Pleasant Prairie, Wisconsin) (Fig. 1). For each box, the top and front sides were open and drainage holes were drilled in the bottom. Each top was covered with a piece of galvanized steel hardware cloth (1.3 cm grid size) secured to the top edge of each box using plastic cable ties. Each branch was arranged horizontally, bottom side down, on the hardware cloth by securing the plastic film canister to the hardware cloth with a cable tie. An adhesive card (7.6 × 17.8 cm) cut from a Delta Trap insert (Alpha Scents Inc., West Linn, Oregon) was placed sticky-side up in the bottom of each box, 5 cm below the branch (Fig. 1) The boxes were screwed to low rectangular platforms (244 × 61 × 4 cm) constructed from plywood and pressure-treated landscape timbers. Four boxes were spaced 61 cm apart on each of eight platforms, which served as the experimental blocks in a randomized complete block design. Blocks were spaced 2 m apart.

The four branch tips per platform were randomly assigned to four sunlight filtering treatments. Filters were constructed of rigid acrylic sheets (40.6 × 30.5 × 0.3 cm, distributed by Ridout Plastics Co., San Diego, California) suspended horizontally, 10 cm above the branches, on two parallel pieces of aluminium trim channel (244 × 1.3 × 1.3 cm) (The Hillman Group, Cincinnati, Ohio) mounted on wooden supports (Fig. 1). The four filter treatments were as follows:

1. Full sun (no acrylic sheet, no wavelengths blocked).
2. Clear-UV filter. A clear, transparent, combustible thermoplastic rated to block light wavelengths below 400 nm (OP3/UF-5, Optix®-UVF Plaskolite, Inc., Columbus, Ohio).
3. Amber filter. An amber-coloured, transparent combustible thermoplastic rated to block light wavelengths below 540 nm (Chemcast® GP acrylic sheet colour 2422).
4. Opaque. Acrylic sheet with paper masking on both sides and painted on the top side with white exterior latex paint, intended to block all direct sunlight.

Branches and filters were deployed from 30 March 2018 to 13 April 2018 in an open field in Buncombe County, NC (35.7596° N, −82.5874° W). The minimum, mean, and maximum temperature during the March-April trial was −1.7 °C, 9.6 °C, and 25.0 °C, respectively, as recorded at the nearest weather station for which hourly data were available (NCDC, 2018). Adhesive cards were collected and replaced 3, 7 and 14 days after the start of the experiment, and the hydrated hemlock branch tips were collected at 14 days. The number of *A. tsugae* progrediens crawlers per cm that settled on the top and bottom sides of the hemlock branch, and the number of crawlers that fell onto each adhesive card, were counted using a stereomicroscope.

June trial. The second trial was conducted from 4 to 8 June 2018. The methodology was identical to the first trial with the following differences. Infested *T. canadensis* material was collected from at the Bent Creek Experimental Forest (35.4916° N, −82.6300° W), the mean (SE) number of sistens eggs per ovisac was 26 (2), and 20 ovisacs were attached to each hydrated experimental branch. In order to evaluate branches that were physically sheltered from above, but still exposed to ultraviolet light, a clear-UV-transmitting treatment was added to the original four treatments in each experimental block. This treatment utilized a rigid stabilized ultraviolet-selective transmitting acrylic sheet (40.6 × 30.5 × 0.4 cm) designed to allow transmission of a high percentage of visible and ultraviolet light in both the UVA (315–400 nm) and UVB (280–315 nm) regions (Item PATTCLR0.187OP4, Ridout Plastics Co., San Diego, California). Branches and filters were deployed from 4 to 8 June 2018. The minimum, mean and maximum temperature during the period was 8.3 °C, 19.1 °C and 28.9 °C, respectively, with no precipitation, clear to partly cloudy skies and light winds 0–7 km/h (NCDC, 2018). Presumably due to the higher temperatures experienced in June, some of the hydrated branches began to show signs of desiccation by the fourth day of the trial. The June trial was therefore terminated earlier than the March–April trial (4 d vs 14 d) and the proportion of each shoot visibly desiccated (characterized by brown shoot tips and needle loss) was recorded along with the number of *A. tsugae* crawlers on the branches (top and bottom sides) and adhesive cards.

To validate the filtering capacity of each treatment, the amount of light transmitted through each of the acrylic sheet products was evaluated by two methods as detailed in the Supplementary Material. Both the transmittance of each acrylic filter material (Fig. S1) and the ultraviolet light intensity measured beneath it (Table S1) were consistent with the manufacturer's technical specifications.

Data analysis

Shade cloth experiment. Mixed-model analysis of variance (ANOVA) was performed using the PROC MIXED procedure of SAS version 9.4 (SAS Institute Inc, 2012) to evaluate the

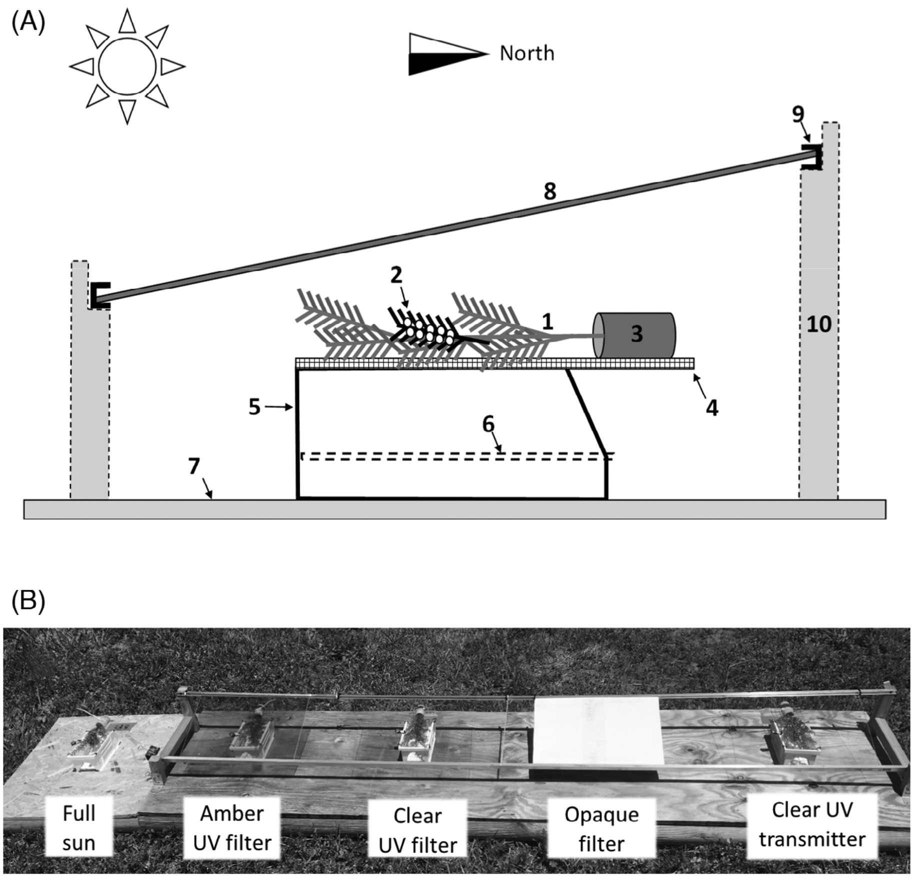

Figure 1 Profile view of the arena used to assess *A. tsugae* crawler response in the acrylic filter experiment (A): uninfested hemlock branch (1), branch segment infested with *A. tsugae* ovisacs (2), film canister containing hydrated floral foam (3), galvanized steel hardware cloth (4), plastic box with open top and interior (5), adhesive card to catch crawlers dropping inside the box (6), plywood platform (7), acrylic sheet filter treatment (8), aluminum trim channel to suspend filters (9), wooden support for trim channel (10). Overhead view of a block of five treatments in the June trial of the ultraviolet filter experiment (B).

effects of the shade treatments on *A. tsugae* density, separately for each of the five data collection time points. Shade treatment was considered a fixed effect, whereas block was considered a random effect. Denominator degrees of freedom were calculated using the Kenward-Rogers method (DDFM = KR in the MODEL statement) (Kenward & Roger, 1997). *F*-tests for fixed effects were considered significant when $P < 0.05$ and means separation was performed using Tukey-Kramer HSD. Analysis of pre-treatment May 2012 progrediens adult density used untransformed data, whereas other data sets were square root transformed (November 2012 sistens nymphs) or $\log_{10}$ transformed to reduce heteroscedasticity. Although transformation improved data normality for the February, June and November 2013 data sets, it did not fully correct for issues related to variance heterogeneity. Therefore, the PROC MIXED ANOVA model for these data sets included the REPEATED/GROUP=SHADE statement that allowed error variance to be estimated separately for each shade treatment.

Acrylic filter experiment. In the March-April trial, mixed-model ANOVA was used to test the null hypothesis that the mean total number of *A. tsugae* crawlers recovered in the experimental arena (crawlers that settled on the branch, plus crawlers that dropped onto the adhesive card; used to indicate the number of crawlers produced) did not differ by filter treatment. Filter treatment was considered a fixed main effect, whereas block was modelled as a random effect. Three additional response variables (the proportion of recovered crawlers that settled on the branch, the number of crawlers per cm settled on each branch, and the proportion of settled crawlers attached to the bottom side [*vs*. top side] of the branch) were analysed similarly, but with the total number of crawlers recovered added to the model as a covariate. These same models were also applied to the data in the June trial, but with the addition of another variable, the proportion of branch desiccated, as a second covariate in each model. Also in the June trial, the proportion of branch desiccation was analysed as a response variable and modelled as a function of filter treatment (fixed main effect), block (random effect) and total number of crawlers recovered (covariate). All analyses were run in the Fit Model procedure in JMP® 14.0.0 (SAS Institute Inc., 2018) using a Standard Least Squares approach and the Restricted Maximum Likelihood method. *F*-tests for fixed effects were considered significant when $P < 0.05$ and means separation was performed using Tukey-Kramer HSD.

Table 1 Analysis of variance (ANOVA) statistics for the effect of shade cloth treatment (0%, 40% and 80% shade) on hemlock woolly adelgid density on potted hemlock seedlings, by assessment date

Assessment date	Adelgid generation and life stage	*F*	df	*P*
May 2012 (pre-treatment)	Progrediens adults	0.02	2, 8	0.984
November 2012	Sistens nymphs	59.39	2, 8	<0.001
February 2013	Sistens adults	119.73	2, 16	<0.001
June 2013	Progrediens adults	109.63	2, 4	<0.001
November 2013	Sistens nymphs	67.95	2, 49	<0.001

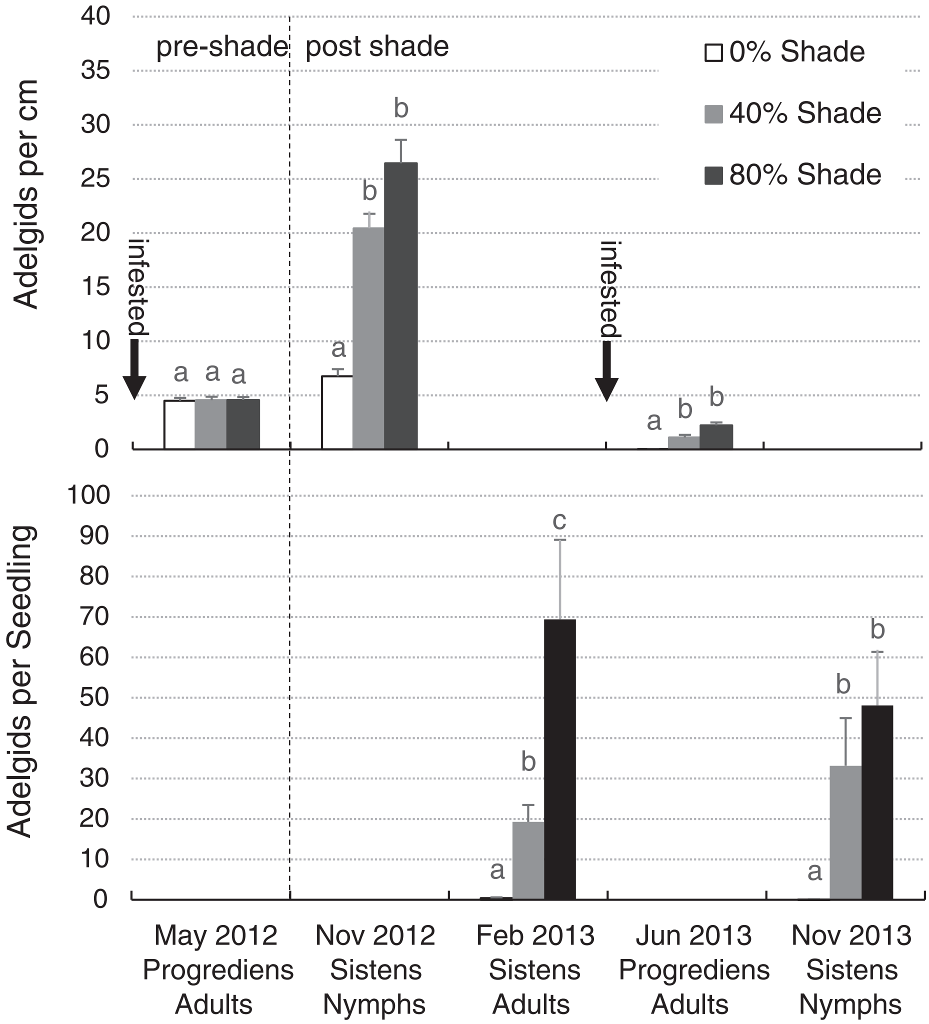

Figure 2 Mean number of hemlock woolly adelgids per cm (top panel) and per seedling (bottom panel) on potted eastern hemlock seedlings exposed to different levels of shade. Arrows indicate manual infestation with *A. tsugae* in March 2012 and March 2013. Dashed line indicates placement of seedlings in treatments immediately after assessment of progrediens density in May 2012. Bars indicate standard error and columns within a sampling period labelled with the same letter are not significantly different ($\alpha = 0.05$).

Results

Shade cloth experiment

In May 2012, immediately prior to placement of the infested seedlings into the shade tents, *A. tsugae* progrediens ovisac density did not differ by shade treatment group, as intended (Table 1 and Fig. 2). In contrast, there was a significant effect of shade treatment on *A. tsugae* density at all four post-treatment data collection time points (Table 1). When settled nymphs of the ensuing sistens generation were evaluated in November 2012, mean densities in the 40% and 80% shade treatments (20 and 26 adelgids/cm, respectively) did not differ from each

Table 2 Statistics for analyses of variance and covariance evaluating the main effect of sunlight filter treatment (Filter) and two covariates (TNR = total nymphs recovered, and PBD = proportion branch desiccated), when applicable, on hemlock woolly adelgid nymph and branch desiccation variables

		March–April trial			June trial		
Response variable	Effect	*F*	df	*P*	*F*	df	*P*
Total nymphs recovered (TNR)	Filter	5.66	3, 21	**0.005**	8.78	4, 28.2	**<0.001**
	PBD	—	—	—	0.87	1, 32.9	0.358
Proportion of recovered nymphs settled on branch	Filter	3.25	3, 20.7	**0.043**	48.4	4, 28.4	**<0.001**
	TNR	2.88	1, 25.6	0.102	1.45	1, 32.9	0.237
	PBD	—	—	—	0.04	1, 32.1	0.837
Settled nymphs per cm of branch	Filter	2.82	3, 20.8	0.064	9.02	4, 27.3	**<0.001**
	TNR	0.22	1, 24.7	0.647	28.85	1, 32.0	**<0.001**
	PBD	—	—	—	0.72	1, 29.9	0.404
Proportion of settled nymphs on bottom side of branch	Filter	22.55	3, 21.4	**<0.001**	2.55	4, 28.6	0.060
	TNR	0.32	1, 26.8	0.578	0.84	1, 33.0	0.367
	PBD	—	—	—	1.92	1, 31.8	0.176
Proportion of branch desiccated (PBD)[a]	Filter	—	—	—	7.03	3, 20.4	**0.002**
	TNR	—	—	—	1.14	1, 20.4	0.298

[a]Analyses involving the proportion of branch desiccated (PBD) were not performed for the March–April trial because all mean and variance values were zero (no branch desiccation). In the June trial, data for the opaque filter treatment were excluded because the mean and variance were zero. The significant bold values for $P \leq 0.05$.

other, but were three times higher than the density observed on unshaded seedlings (7 adelgids/cm) (Fig. 2). These sistens nymphs had not produced wool by November 2012. When the seedlings were reassessed in February 2013, very few adelgids had survived to produce woolly ovisacs, so whole-seedling adult counts were used. In February 2013, mean whole-seedling sistens adult counts in 80% shade (69 adelgids/seedling) were more than three times the density observed in 40% shade (19 adelgids/seedling), whereas mean adult counts on unshaded seedlings were nearly zero (0.4 adelgids/seedling) (Fig. 2). After attempting to re-infest all seedlings with progrediens crawlers in March 2013, adult densities of the next two generations continued to be significantly higher in the shade treatments compared to unshaded seedlings, in which densities remained near zero (Fig. 2).

Acrylic filter experiment

March–April trial. In the March-April trial, the mean total number of *A. tsugae* crawlers recovered in the experimental arena (those that dropped onto the adhesive card plus those that settled on the branch) differed by filter treatment (Table 2). Significantly more crawlers were recovered under the opaque and amber filters than beneath the full sun treatment (Fig. 3(A)). The total number of crawlers recovered beneath the clear-UV filter was fewer than that recovered beneath the opaque filter, but did not differ from the full sun or amber filter treatments (Fig. 3(A)). The mean proportion of all recovered crawlers that settled on the branch (as opposed to dropping) also differed by filter treatment (Table 2). A higher proportion of crawlers settled on the branch beneath the opaque filter than beneath full sun, whereas the proportion that settled beneath the amber and clear-UV filter treatments were intermediate and did not differ significantly from other treatments (Fig. 3(B)). Although the mean number of crawlers settled per cm of branch was more than twice as high beneath the opaque and amber filter as beneath the clear-UV filter and full sun treatments, these differences were not significant with the inclusion of the total number of crawlers recovered as a covariate in the model (Table 2 and Fig. 3(C)). The mean proportion of settled crawlers that attached to the bottom side (vs. top side) of the branch was significantly higher beneath the clear-UV filter and full sun treatments (40%) than beneath the opaque (25%) and amber filter (14%) (Table 2 and Fig. 3(D)). There was no evidence of branch desiccation in the March-April trial.

June trial. In the June trial, the mean total number of *A. tsugae* crawlers recovered in the experiment again differed by filter treatment (Table 2). Significantly more crawlers were recovered under the opaque filter than beneath all other treatments (Fig. 3(A)). The mean proportion of crawlers that settled on the branch was three times higher beneath the opaque filter (73%) than beneath the amber filter (24%), which in turn was approximately three times higher than beneath the clear-UV filter, clear-UV transmitting sheet and full sun treatments (Table 2 and Fig. 3(B)). Similarly, the mean number of crawlers settled per cm of branch was significantly higher beneath the opaque filter (2.8 nymphs/cm) than beneath the other treatments (≤0.5 nymphs/cm) (Table 2 and Fig. 3(C)). A covariate variable, the total number of crawlers recovered, was also significant in the model for the density of crawlers settled per cm (Table 2). Unlike the March-April trial, the proportion of settled crawlers that attached to the bottom side (vs. top side) of the branch did not differ significantly by filter treatment in the June trial (Table 2 and Fig. 3(D)). Branches in the June trial exhibited evidence of shoot tip desiccation beneath all treatments except the opaque filter, and the proportion of branch desiccated varied significantly by treatment (Table 2). After just 4 days in the experimental arena, the mean (SE) proportion of branch desiccation was significantly higher beneath the clear-UV filter [53 (7)%] and clear-UV transmitter [61 (11)%] treatments than beneath the amber filter [9 (8)%] or full sun [17 (5)%] treatments.

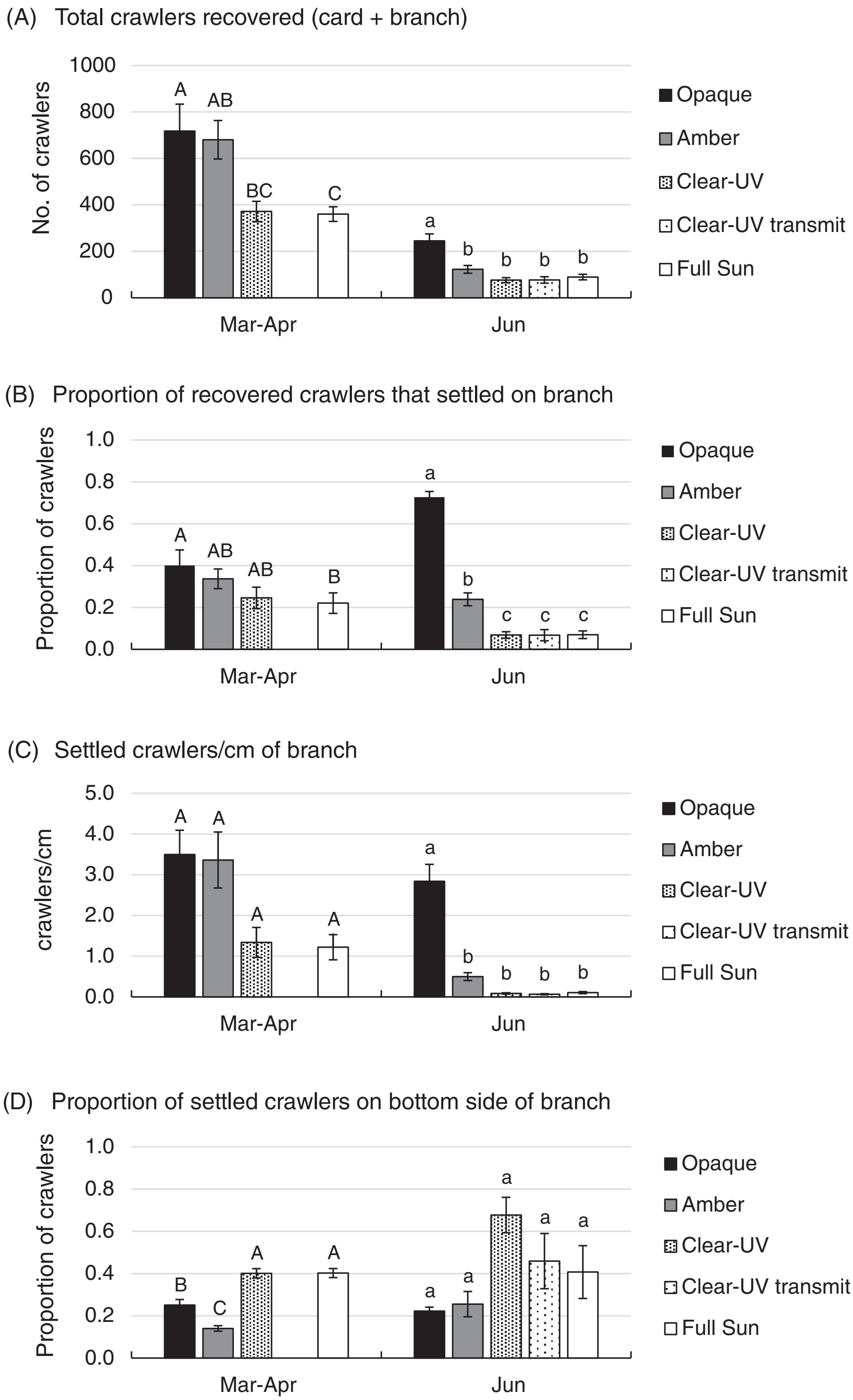

Figure 3 Mean responses of *A. tsugae* progrediens (March–April trial) and sistens (June trial) crawlers beneath various sunlight filter treatments in 2018: total number of crawlers recovered on adhesive card plus those settled on branch (A), proportion of recovered crawlers that settled on the branch (B), number of crawlers per cm settled on branch (C), and proportion of settled crawlers attached to bottom (vs. top) side of branch (D). Within each trial, means labelled with the same letter are not significantly different ($\alpha = 0.05$). Bars indicate standard error of the mean. The clear-UV transmitter treatment was not included in the March-April trial.

Discussion

Shade cloth experiment

The results of the shade cloth experiment demonstrated that elevated levels of sunlight can dramatically decrease the number of *A. tsugae* crawlers settling on eastern hemlock seedlings. This is consistent with other studies that have demonstrated a positive relationship between adelgid density and increasing application of shade (Hickin & Preisser, 2015; Brantley *et al.*, 2017, McAvoy *et al.*, 2017). Our shade cloth study differed from those of Hickin and Preisser (2015) and Brantley *et al.* (2017) in that our seedlings were already infested with equal densities of mature progrediens adults before the initial shade treatments were applied in May. Because the progeny from these ovisacs (sistens crawlers) disperse and settle in May and June (immediately after shade was introduced), the dramatically lower densities of settled sistens nymphs observed in the next generation in the full sun treatment were likely due to direct, negative phototactic or thermotactic effects of sunlight on the crawlers as they dispersed, rather than by effects mediated through the host plant after crawlers settled.

Both negative phototactic and negative thermotactic behaviour have been observed in the closely related species balsam woolly adelgid, *Adelges piceae* (Ratzeburg), whose crawlers dropped from host material in synchrony with increases in both light and heat (Atkins & Hall, 1969). Negative phototaxis has also been observed in other groups of insects including haematophagous bugs (Lazzari *et al.*, 1998) and stored grain beetles (Kim *et al.*, 2013). As noted by McAvoy *et al.* (2017), hemlocks are very shade-tolerant and frequently grow in the forest understory, so if *A. tsugae* is adapted to cool, shaded environments, sunlight avoidance may help them locate cooler settling sites. This hypothesis is consistent with experiments by Mech *et al.* (2018), who documented an increase in the mortality of aestivating *A. tsugae* sistens nymphs with increases in temperature, a relationship that strengthened with increasing duration of exposure, particularly above 30 °C. Sussky and Elkinton (2015) also reported high levels of aestivating sistens mortality associated with elevated temperatures.

Notably in our shade cloth experiment, sistens nymphs that settled in May–June of 2012 had not broken aestivation (i.e. had not begun to feed or produce wool) by November 2012 in any of the treatments. In the southern Appalachians, the sistens generation usually breaks aestivation by late September or early October (Joseph *et al.*, 2011). Mean July maximum daily temperature at the study site (1950–2019) was 28.2 °C, but July 2012 included 11 consecutive days during which the maximum temperature exceeded 30 °C, peaking at 35.6 °C on 1 July (NCDC, 2018). This period of extreme heat is suspected to have killed most of the settled sistens nymphs and also delayed the breaking of aestivation among those that survived. Results indicate that the shade treatments differentially affected this summer mortality: although the 40% and 80% shade treatments had similar densities of settled sistens nymphs in November 2012, the number of surviving sistens (woolly adults) per seedling in February 2013 was three times higher beneath 80% shade than beneath 40% shade, and was nearly zero in full sun (Fig. 2).

Although the initial densities of sistens nymphs in this study were likely driven by direct effects as described above, our seedlings remained in the shade treatments for 18 months. Thus, subsequent generations of adelgids may also have been influenced by longer-term, indirect effects of sunlight mediated through the host plant. In artificial shade studies by Hickin and Preisser (2015) and Brantley *et al.* (2017), eastern hemlock seedlings were exposed to shade treatments for several weeks or months prior to infestation, during which time host plants experienced sunlight-related effects on host physiology and chemistry. Hickin and Preisser (2015) found that shade increased the relative water content and water potential of eastern hemlock seedlings, and depending on time of measurement, decreased rates of transpiration and photosynthesis. Measuring the same experimental seedlings reported in Brantley *et al.* (2017), Lapham *et al.* (2018) showed that after 9 months, hemlock foliar nitrogen concentration increased positively with increasing shade, and this relationship strengthened under the extended influence of both shade and introduced adelgids. Furthermore, Brantley *et al.* (2017) demonstrated heavily shaded hemlock seedlings had reduced foliar starch content 4–6 months after the introduction of adelgids compared to seedlings in full sun. One possible reason that seedlings exposed to 10 months of full sun in the present study were not successfully re-infested in March 2013 is that sunlight-related chemical changes to these seedlings made them less susceptible to infestation. Future studies designed to differentiate the relative importance of direct versus indirect effects would help elucidate the relationship between sunlight and adelgid infestation.

Acrylic filter experiment

In the acrylic filter experiment, equal numbers of *A. tsugae* ovisacs (containing mature eggs) were manually attached to each hydrated branch, with the intention of exposing each branch to similar numbers of hatching crawlers. It was therefore assumed that the total number of crawlers recovered (those that settled on the branch plus those that fell onto the adhesive card) would be similar across treatments, and that the treatments would differentially influence the proportion of crawlers that settled versus dropped. However, more crawlers were recovered beneath treatments that transmitted the least amount of light, namely the opaque filter (in both trials) and the amber filter in the March-April trial (Fig. 3(A)). This suggests that solar radiation affected the total number of crawlers that hatched and dispersed from the manually applied ovisacs, independent of treatment effects on settling behaviour.

One likely mechanism for this unanticipated effect is that the nonhydrated, infested twig segments (which were tied to the hydrated branches) may have desiccated more quickly beneath the clear filter and full sun treatments compared to the opaque and amber filters, possibly due to elevated temperatures at the branch surface. Although the number of ovisacs and eggs on these twig segments were similar across treatments at the time they were applied, adults may have continued to produce eggs after deployment, but at a reduced rate on twigs exposed to a wide spectrum of solar radiation. A second possible mechanism by which fewer crawlers were produced beneath the clear-UV filter, clear-UV transmitter and full sun treatments is that more eggs died within the manually applied ovisacs in these treatments. Irradiation with ultraviolet or short-wave visible light (violet-blue) has been demonstrated to cause egg and/or pupal mortality in certain species of insects (Beard, 1972; Hori *et al.*, 2014). This second mechanism may be less important for a species like *A. tsugae*, however, because eggs are laid within the waxy material of the ovisac, which likely provides some protection from solar radiation.

Despite variable crawler production across the treatments, the acrylic filter experiment provided evidence that sunlight directly affects *A. tsugae* crawler settling behaviour. After accounting for total crawler production (by adding it as a covariate to the model), the proportion of crawlers that settled on the branch (*vs*. dropping from it) was higher beneath the opaque filter (both trials) and the amber filter (June trial) than beneath the full sun treatment (Fig. 3(B)). Furthermore, in the March–April trial, treatments providing exposure to full sun and the full visible spectrum only (clear-UV filter) significantly increased the proportion of crawlers that settled on the bottom side of the hemlock branch (Fig. 3(D)). These trends are consistent with the results of the shade cloth experiment and further suggest a negative phototactic or thermotactic response in *A. tsugae* by which crawlers navigate away from sunlight.

Our results suggest that treatment effects on crawlers in the acrylic filter experiment were mediated primarily by exposure to sunlight in the visible spectrum (400–780 nm) rather than the ultraviolet (UVA and UVB, 280–400) spectrum. The amber filter and clear-UV filters blocked similar amounts of UVA/UVB radiation (Table S1), but the amber filter also blocked additional wavelengths in the lower end of the visible spectrum (400–540 nm) that were transmitted through the clear-UV filter (Fig. S1). In the March–April trial, crawlers were more likely to settle on the bottom side of the hemlock branch beneath the clear-UV filter as compared to the amber filter (Fig. 3(D)). These two treatments also differed in terms of the proportion of crawlers settled in the June trial (Fig. 3(B)). Furthermore, none of the crawler response means differed between the clear-UV filter (which blocked UV light) and the clear-UV transmitter or full sun treatments (both of which transmitted UV light) in either trial (Fig. 3), again suggesting that the filtering of ultraviolet light was unimportant to the patterns observed.

Notably, the only response variable that differed between the full sun treatment and either of the clear acrylic sheet treatments (UV filter and UV transmitter) was the proportion of branch desiccation in the June trial. More than half of the cumulative hemlock shoot length showed desiccation symptoms beneath the clear-UV filter and clear-UV transmitting sheets, whereas branch desiccation in the full sun treatment was less than 20% and did not differ significantly from the amber filter treatment. This suggests that in the June trial (during which the mean local temperature was 9.5 °C warmer than in March-April), the clear sheet treatments created a greenhouse effect in which radiant heat was trapped beneath the acrylic canopy. An attempt was made to minimize this effect by leaving all vertical sides of each treatment arena open to the air, but evidently, it was not eliminated. Despite this effect, branch damage was not a significant covariate in any of the analyses examining the relationship between crawler behaviour and sunlight filter treatment (Table 2).

In summary, these results indicate that exposure to sunlight directly and negatively affects the settling behaviour of *A. tsugae* crawlers. We propose that this direct effect of sunlight on the insects acts in combination with indirect effects mediated through the host plants (Hickin & Preisser, 2015; Brantley *et al.*, 2017, Miniat *et al.*, 2020) to benefit infested eastern hemlocks. Collectively, these and other studies (McAvoy *et al.*, 2017; Lapham *et al.*, 2018) indicate that silvicultural treatments to increase the amount of ambient light in stands containing hemlock could be a useful tool in an integrated pest management strategy for hemlock woolly adelgid. Although eastern hemlocks are extremely tolerant of shade, advance regeneration responds well to increased light created by the formation of small gaps or moderate basal area removals (Goerlich & Nyland, 2000). Recent (Miniat *et al.*, 2020) and ongoing (Mayfield *et al.*, 2019) research on the effect of specific silvicultural treatments on adelgid densities and eastern hemlock health indicate promise for this approach.

Acknowledgements

Sincere thanks to Andrew Tait (formerly North Carolina State University), Andrew Whittier (North Carolina State University), and Bryan Mudder (USDA Forest Service Southern Research Station) for field and laboratory assistance. Thanks also to Kaleb Rathbone and the North Carolina Department of Agriculture and Consumer Services/North Carolina State University Mountain Research Station for use of facilities. Daniel Miller (USDA Forest Service Southern Research Station) and Nathan Havill (USDA Forest Service Northern Research Station) provided helpful reviews of an early draft of the manuscript. This research was funded by the USDA Forest Service Southern Research Station, the USDA Forest Service Forest Health Protection Region 8, and Camcore—North Carolina State University. The use of trade names and identification of firms or corporations does not constitute endorsement or approval by the United States Government or Camcore—North Carolina State University of any product or service to the exclusion of others that may be suitable.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Percent light transmission measurements (at wavelengths 380–700 nm) through four types of acrylic filter treatments used to examine sunlight effects on *A. tsugae* crawler behaviour.

Table S1. Mean (SE) intensity of ultraviolet light (UVA/UVB) measured beneath full sun and acrylic sheet filter treatments during March and June 2018 in Buncombe County, NC.

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Accepted 27 April 2020