BRIEF COMMUNICATION

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Shade and Hemlock Woolly Adelgid Infestation Increase Eastern Hemlock Foliar Nutrient Concentration

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Tsuga canadensis (L.) Carr. (eastern hemlock) is dying across much of eastern North America from the invasive hemlock woolly adelgid (HWA, *Adelges tsugae* Annand). Survey studies show that eastern hemlock populations with high foliar nutrient concentrations are associated with low infestation rates, and also suggest that deeply shaded trees may be more susceptible to infestation. Here we examined (1) how foliar nutrient concentration of eastern hemlock changes with varying shade levels; and (2) how nutrient concentration might further change with sustained shade and subsequent HWA infestation. Foliar samples from three years—pretreatment, post-shade, and post-shade and infestation—were collected and analyzed for [N], [P], and [K]. Pretreatment, all seedlings had similar foliar nutrient concentrations. After nine months in the shade tents, seedlings under higher levels of shade exhibited increased foliar [N]. For each 10% increase in shade, foliar [N] increased 35.09 μg/mg over baseline levels. The combined effects of prolonged shade with HWA infestation increased foliar [N], [P], and [K]. The mechanism for increasing foliar nutrients is unknown, but may be due to reduced growth causing a concentration effect, or nutrients mobilized by the plant in response to infestation.

Keywords: Adelges tsugae, eastern hemlock, foliar nutrient content, invasive species, shade

Tsuga canadensis (L.) Carr. (eastern hemlock) dominates about 1 million ha of forest in eastern North America (McWilliams and Schmidt 2000), yet, trees of all sizes and ages throughout its range are dying due to an invasive insect, the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand) (Orwig et al. 2002). In the United States, HWA produces two asexual generations each year—a sistens generation in winter and a progrediens generation in spring and early summer. Resistance to HWA in eastern hemlock is rare (Oten et al. 2014), and tree mortality can be rapid (Ford et al. 2007).

How abiotic factors—such as light, temperature, and nutrient availability, and their interactions—influence eastern hemlock susceptibility to HWA is largely unknown. Recent studies suggest that both high light (Mayfield and Jetton 2013, Hickin and Preisser 2015) and temperatures (Mech 2015, Sussky and Elkinton 2015) regimes may negatively affect HWA success. High foliar nutrient content, particularly nitrogen, has been shown to increase the fecundity of aphid-like insects (McClure 1980, 1992, Stadler et al. 2005, Joseph et al. 2011, Jones et al. 2015). In contrast, high phosphorus has been shown to limit herbivore success, but generalities are elusive, as effects are plant/host specific or study designs do not directly test this (Hawkins et al. 1986, Schade et al. 2003, Pontius et al. 2006).

A recent study using potted eastern hemlock seedlings demonstrated that elevated light levels (i.e., less artificial shade)

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reduced HWA densities and improved plant carbon balance of infested eastern hemlock seedlings (Brantley et al. 2017). During the course of that experiment, we observed large differences in the infestation success among shade treatments. We speculated that it might have been related to foliar chemistry and established the present companion study, which analyzed pre-harvested foliar samples for [N], [P], and [K] to see if there were relationships with shade and HWA densities. While the study design is not ideal (see below), we report our results here as a brief communication that may lend supporting evidence for a wider trend to be more fully explored with future studies. The present study aimed to examine how foliar nutrient concentrations of eastern hemlock (1) change with shade, and (2) change with prolonged shade with subsequent HWA infestation. We hypothesized that: (1) pretreatment seedlings would have similar foliar nutrient investment; (2) shade would influence foliar nitrogen, phosphorus, and potassium concentrations; and (3) HWA densities would follow patterns of foliar nutrient investment.

Materials and Methods

Study Area, Experimental Design, and Shade Treatments

The study area, experimental design, shade treatments, and HWA infestation treatments are described fully in Brantley et al. (2017) and briefly summarized here. The study was conducted in Waynesville, NC, USA (35.48752°, –82.96768°, elevation 820 m, mean annual temperature 12.8 ºC). Twenty shade tents (1 m x 1 m x 1.3 m) arranged in four rows were established in an open field. Tent frames were randomly assigned a treatment and covered with shade cloth to achieve either 0 (no shade cloth), 30, 50, 70, or 90% shade. In May 2013, 100 four-year-old bare root eastern hemlock seedlings were potted in 6-liter plastic pots in a growing mix with 15 g of 15-16-17 fertilizer. On 12 July 2013, five potted seedlings were placed in each shade tent. Twice per week in spring, summer, and fall, seedlings were drip irrigated at 1.9 liters of water per hour for 2–4 hours. Irrigation was adjusted for low-rainfall periods as needed (Brantley et al. 2017).

Infestation with *A. tsugae*

After nine months in the shade treatments, all seedlings were artificially infested with HWA. We note that this study design does not have proper controls that were (1) shaded, and (2) unshaded but not infested; thus, we cannot completely disentangle the effects of shade from HWA on foliar chemistry. Eastern hemlock branches infested with HWA sistens ovisacs and progrediens eggs were cut into 40-cm-long pieces and cut ends placed in water until used. Heavily and lightly infested 40-cm shoots averaged 257 and 108 ovisacs per shoot, respectively, whereas both groups averaged 15 eggs per ovisac. Four shoots (two heavily and two lightly infested) were loosely attached to each seedling using metal paper clips (Jetton et al. 2014), resulting in an estimated 11,000 progrediens eggs applied per seedling. Progrediens crawlers (first instars that emerge with functional legs) hatched and dispersed from source branches after approximately 3 weeks.

The HWA progrediens generation settled on the seedling's 2013 shoot growth and gave rise to a sistens generation that settled on the 2014 shoot growth. Ovisac counts of the settled progrediens and sistens generations were made on June 9, 2014, and January 21, 2015, respectively. Counts were made on five 10-cm shoot tips (one terminal and four laterals) and averaged to produce a mean density (HWA dm^{-1}) per seedling, as originally reported in Brantley et al. (2017).

Foliar Analysis

Foliage was collected from terminal branches from each seedling at three sampling times: t1, July 12, 2013, immediately prior to imposing shade treatments; t2, March 19, 2014, after 9 months of shade treatment and immediately prior to infestation; and t3, April 2, 2015, after 11 months of continued shade and infestation (Figure 1). Samples were transported to the lab on ice, and frozen until processed. Samples for t1 and t2 were dried at 60°C for three days and then ground (8000D MixerMill). Samples collected in t3 were dried at 100°C for one hour prior to drying at 60°C for three days. Samples from t3 were processed differently because they were also analyzed for carbohydrates (Brantley et al. 2017). All dried and ground samples were then analyzed for N (Thermo Electron Corp. Flash EA 1112 NC) following the Dumas method, and for P and K (Thermo Scientific iCAP 6300 Inductively Coupled Plasma Optical Emission Spectrometer) following the dry ash method (Miniat and Brown 2017).

We tested whether drying temperature affected foliar nutrient concentration, by analyzing foliage collected from 10 eastern hemlock trees growing in the Coweeta Basin, NC, along an N availability gradient. Samples and a certified reference (NIST peach leaves SRM#1547) were treated and analyzed as described above for t1/t2 and t3. A paired t-test showed that drying temperature did not affect nutrient concentration (for N, P, and K, respectively, $t_8 = 0.88$, $P = 0.40$; $t_8 = -0.07$, $P = 0.95$; $t_8 = -0.86$, $P = 0.41$). Tree 5 was excluded because of suspected contamination.

Statistical Analysis

We modeled foliar [N], [P], and [K] as linear functions of shade and HWA density, as shade was a continuous variable rather than categorical. For all analyses, tent was the experimental unit ($n = 20$); subsamples within tent were averaged. HWA densities were log-transformed to reduce heteroscedasticity. To estimate the effect that HWA infestation had on foliar chemistry in addition to the effects of continued shade (t2 *vs.* t3), we calculated the change

Management and Policy Implications

Due to the persistent changes in terrestrial and stream ecosystem structure and function, a wide variety of strategies are being developed and implemented to reduce the effects of *A. tsugae* on eastern hemlock. However, little is known about how abiotic factors—such as light, temperature, and nutrient availability, and their interactions—influence eastern hemlock susceptibility to *A. tsugae*. This study aims to gain a better understanding of the possible mechanisms of hemlock susceptibility to infestation in the field. The inverse relationship between ambient light and foliar nitrogen concentration and adelgid density suggests that *A. tsugae* infestation may be more severe in heavily shaded environments, and that nutrients might be mobilized to the foliage after infestation to create even greater susceptibility in these shaded environments. Silvicultural treatments that increase light incident on the hemlock canopy may reduce susceptibility to infestation.

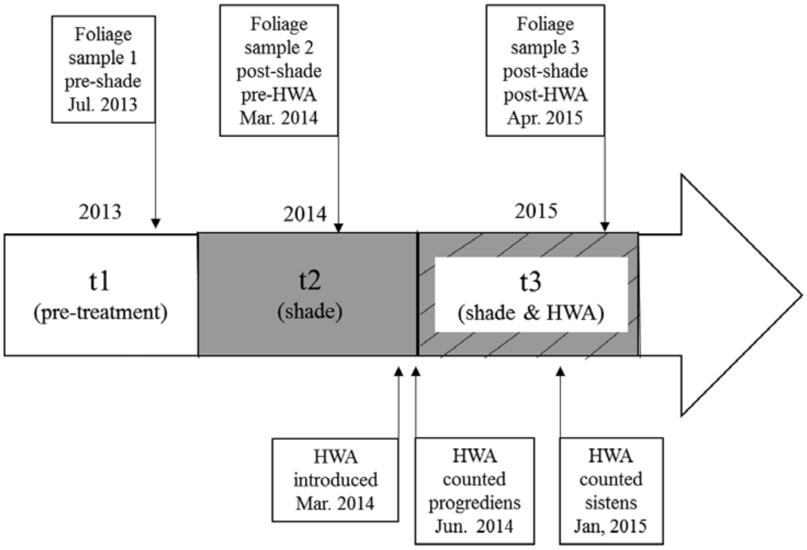

Figure 1. Timeline of shade and *A. tsugae* (HWA) treatment implementation.

in foliar [N], [P], and [K] as 2015 concentration (t3) minus 2014 concentration (t2) and modeled these as linear functions of shade or HWA density.

Results

Prior to shade treatment implementation (t1), mean foliar [N], [P], and [K] did not differ by planned shade treatment group (Figures 2a, 2d, 2g). After nine months in the shade tents, seedlings under higher levels of shade exhibited increased foliar [N] (Figure 2b). For each 10% increase in shade, foliar [N] increased 35.09 μg/mg over baseline levels (Figure 3a). Foliar [P] and [K] were not affected by shade implementation from t1 to t2 (Figures 2e, 2h, 4a). Seedlings under higher levels of shade exhibited higher HWA densities following artificial infestation, in both the settled progrediens (June 2014) and the subsequent sistens (Jan 2015) generations (Brantley et al. 2017). Shade treatments only affected incident light, and did not affect temperature or relative humidity (Brantley et al. 2017).

The combined effects of prolonged shade with HWA infestation (t3) increased foliar [N], [P], and [K] (Figures 2c, 2f, 2i). Foliar [N], [P], and [K] increased at a rate of 115 μg/mg, 0.043 μg/mg, 0.160 μg/mg for every 10% increase in shade, respectively (Figure 2). With each 10% increase in shade, the change in foliar [N] from t2 to t3 increased by 0.079% (Figure 3), and the change in foliar [P] increased by 5.8% (Figure 4). Shade explained variation in [N], and shade and HWA density explained variation in [P] (Figures 3b–c, 4b–c). Shade explained more variation in changes in [K] than HWA density, although relationships were not significant (data not shown).

Discussion

Pretreatment, replicate seedlings had similar foliar N, P, and K concentrations, averaging 1.61%, 0.228%, and 0.734%, respectively. Concentrations are similar to literature reports of healthy eastern hemlock (*ca.* 0.2% [P] and 0.75% [K]), while pretreatment seedling [N] was on the high end of the reported range (*ca.* 1.3%) (Pardo et al. 2005, Niklas and Cobb 2006). After imposing shade, foliar [N] increased with increasing shade, while [K] and [P] did not, which is consistent with literature reports (Tucker and Emmingham 1977, Niinemets 1997, Richardson 2004). In these same seedlings, before infestation, new growth did not differ greatly among shade treatments ≤50% (Brantley et al. 2017). Artificial infestation resulted in higher HWA settlement (HWA density per unit new growth) on shaded seedlings, which had higher foliar [N], than on seedlings in the sun with lower foliar [N]. This suggests that deeply shaded trees with high foliar [N] may be the most susceptible to infestation. After seedlings were infested, leaf [N], [P], and [K] all increased with increasing levels of shade.

Our experiment was not able to discriminate whether HWA settled preferentially in shaded seedlings due to direct effects of light (or UV), or higher foliar [N], or both. The positive relationship between HWA density and shade is consistent with other artificial infestation experiments (Hickin and Preisser 2015), and this pattern may be due to negative phototactic crawler behavior under elevated light (Atkins and Hall 1969). HWA could have also been attracted to foliage with higher N concentrations (Coley 1980, Crawley 1983, Raupp and Denno 1983). Further, once settled, infestation itself seemed to increase foliar [N]. Other studies have found that foliar [N] in infested trees is greater than that of uninfested trees, but the magnitude varies from a substantial 20–40% higher (Stadler et al. 2006, Domec et al. 2013), to a modest *ca.* 4% (Cobb et al. 2006). Recent studies document that foliar N-containing compounds increase with infestation (Gómez et al. 2012, Williams et al. 2016), specifically when HWA is actively feeding (Gonda-King et al. 2014); and that infested saplings have soils with higher N availability, greater uptake of that available soil N, and higher foliar [N] as a result compared to uninfested seedlings (Rubino et al. 2015).

Increasing leaf [P] also may have been more a consequence of infestation rather than shade. Biosynthesis of many defensive compounds (e.g., terpenes, resins) requires P, and an induced defensive response has been documented in eastern hemlock following HWA attack (Radville et al. 2011, Domec et al. 2013, Pezet et al. 2013). Leaf [P] is greater in eastern hemlock trees with poor HWA colonization success (Pontius et al. 2006); and in *Tusga* species that are HWA-resistant compared to susceptible species (Pontius et al. 2006), partially due

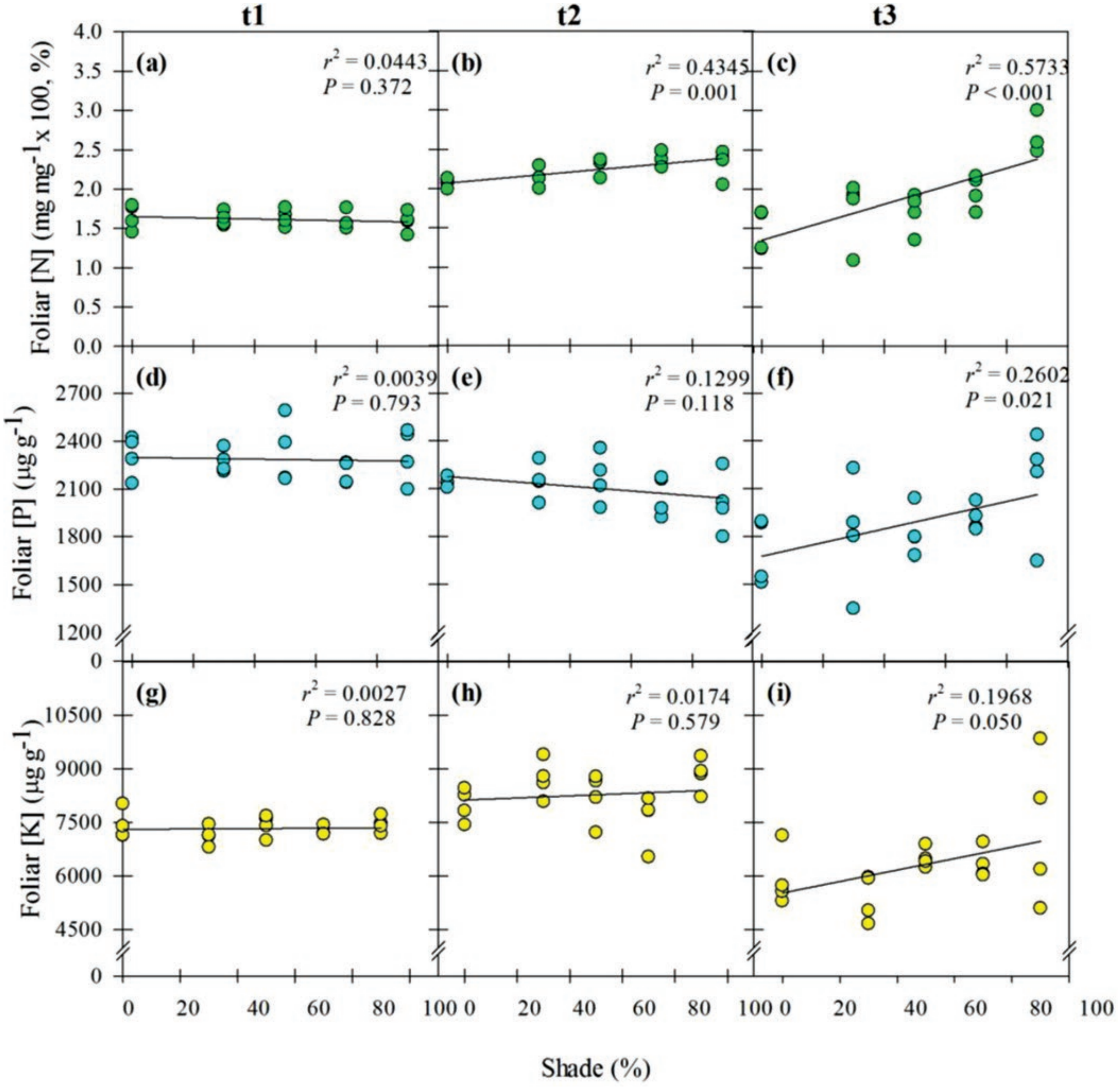

Figure 2. Eastern hemlock foliar nitrogen, phosphorous and potassium concentrations during the study from 2013 to 2015: pre-treatment (a, d, g), after shade implementation (b, e, h), and after shade and *A. tsugae* infestation treatments (c, f, i). Values are plotted against planned level of shade treatment (a, d, g), or imposed shade levels (all other panels). For each panel and shade treatment, *n* = 4 (note: some symbols overlap).

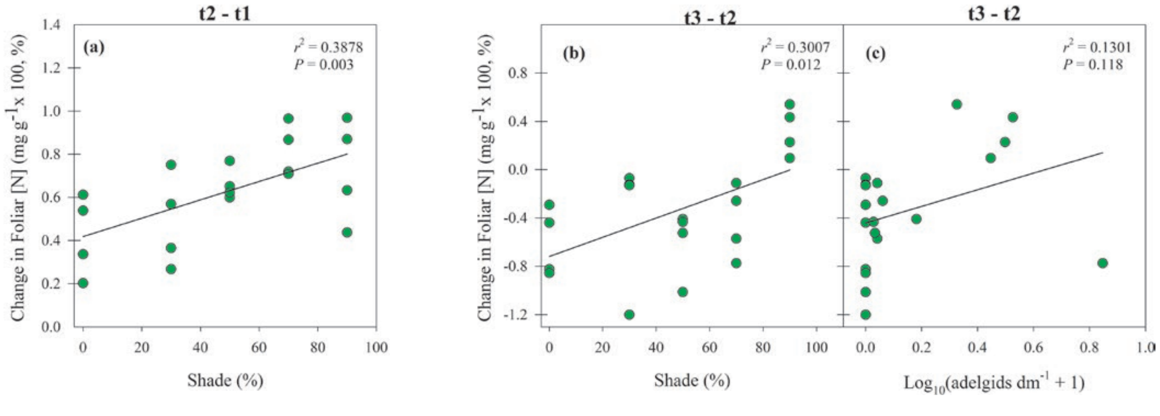

Figure 3. Change in foliar nitrogen concentration attributable to shade implementation, estimated as 2014 [N] measurement minus 2013 [N] measurement (a), and change in foliar [N] attributable to shade implementation and hemlock woolly adelgid (HWA) infestation as a function of shade level (b) and HWA density (c). In (b) and (c), change is estimated as 2015 [N] measurement minus 2014 [N] measurement.

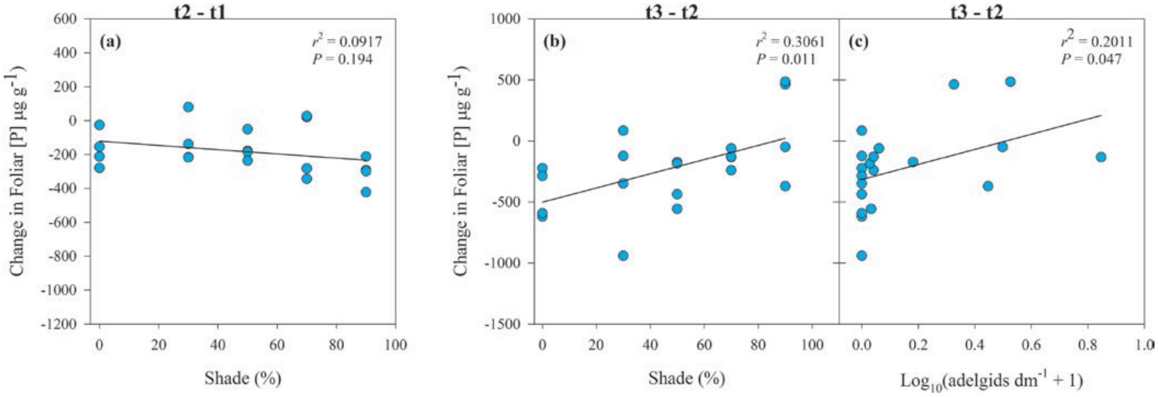

Figure 4. Change in foliar phosphorous concentration attributable to shade implementation, estimated as 2014 [P] measurement minus 2013 [P] measurement (a), and change in foliar [P] attributable to shade implementation and hemlock woolly adelgid (HWA) infestation as a function of shade level (b) and HWA density (c). In (b) and (c), change is estimated as 2015 [P] measurement minus 2014 [P] measurement.

to higher leaf terpene volatiles in the former compared to the latter (Lagalante et al. 2007). While we did not measure defense compounds in our study, the trend toward increase in foliar [P] associated with infestation may have indicated an induced defensive response.

Over the long term, the amount of new growth decreased with HWA infestation (Brantley et al. 2017), consistent with literature findings (Coates and Burton 1999). Decreased new growth in shaded and infested seedlings could have contributed to a "concentration effect" per leaf; because all seedlings had similar soil N availability in the potting media, seedlings with more growth could have had lower tissue concentrations because of reduced relative supply. Less growth and carbon fixation in shaded trees may make trees succumb to mortality more quickly after infestation than trees in higher-light regimes, as found by Krapfl et al. (2011).

Our study is one of the first to track foliar nutrient concentrations over time, as seedlings underwent both shade and infestation treatments. Because our study could not isolate whether HWA preferentially settled on shaded trees in direct response to light, or if they preferentially settled on trees because of increased foliar [N], we suggest a follow-up study employing a fertilization and shade factorial design with artificial infestation to help resolve this question. However, if HWA crawlers are more likely to accept and settle on shoots with higher [N], and infestation also increases foliar [N], then this could indicate a positive feedback of the host to future infestation. Finally, future studies that track foliar changes of infested trees should consider also measuring HWA survivorship and fecundity, as longer-term measures of tree susceptibility.

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