TREE-LEVEL RESPONSE TO DROUGHT AND HEMLOCK WOOLLY ADELGID INFESTATION IN EASTERN HEMLOCK TREES

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Abstract. Tsuga canadensis (eastern hemlock) trees provide unique habitat, help to moderate temperatures and nutrient cycling, regulate stream flow, and provide aesthetic beauty to Northeastern forest communities. Since the 1980's, populations of this tree species have been rapidly declining due to infestations of a small, aphid-like exotic insect, *Adelges tsugae* (hemlock wooly adelgid) that feeds on the stored sugars at the base of a tree's needles. The adelgid can kill hemlocks quickly, often within 3 to 4 years, with some populations surviving longer. Studies suggest that the presence of multiple stressors may affect how quickly a tree succumbs to the adelgid. This study examined the interaction of a water deficit on infested trees by observing response measures of tree health on those with and without experimentally imposed drought - using plastic skirts around a tree's trunk to prevent water from saturating the soil and roots. Counts of adelgid density and measures of photosynthesis from trees with and without plastic skirts were compared. Trees experiencing experimental drought and heavier infestation were predicted to have lower photosynthesis rates and lower chlorophyll fluorescence than trees that were not affected by these stressors. No significant response to the "drought" treatment was observed, probably because the treatment had little impact on soil moisture levels. However, chlorophyll fluorescence and net photosynthesis rate tended to decrease as HWA density increased. Understanding the links between drought and infestation will be critical for predicting future forest response to a changing climate.

INTRODUCTION

Tsuga canadensis (eastern hemlock) is an important conifer species in many forests communities in eastern areas of the US, ranging from the northern tips of Georgia and Alabama, westward to Wisconsin, and northward to Canada (Orwig & Foster 1998). With its unique characteristics – shallow rooting systems, the production of acidic and slow-decomposing litter, dense canopy, low annual rates of evapotranspiration, and moderation of temperature from year-round canopy cover – eastern hemlock is a late-successional–foundation species, a primary producer that defines an ecosystem by regulating many ecological processes in its environment (Ellison *et al.* 2005). In addition to structuring the vegetative communities of the forest floor and regulating abiotic factors, hemlocks provide a unique habitat for mammals, birds, and other organisms (Small & Small 2005).

Eastern hemlock-dominated forests are currently facing rapid shifts in forest species composition resulting from the presence of various exotic pests and their influence on ecosystem structure and function. Since midcentury, populations of eastern hemlocks have been decimated by an invasive, aphid-like insect called the Hemlock Wooly Adelgid (abbr. HWA – *Adelges tsugae*), a native to Japan. HWA has spread to roughly 17 states, where populations expand quickly due to lack of native predators; little plant host resistance; and facilitated dispersal by wind, animals, and humans (Orwig & Foster 1998). The HWA has a complex, parthenogenic life cycle; populations can increase rapidly as the insects complete two generations per year. Summer adults, or progrediens, mature in the spring and lay their eggs in a waxy bundle on the underside of a stem. The winged, sexually reproducing adults, sexuparae, also develop at this time but without their obligate spruce host in the United States, they presumably die (DCNR 2011). The sistens generation emerges in mid-summer, undergoes a period of aestivation, and then actively feeds and matures through the following winter. The cycle begins the next spring when the sistens lay their eggs in another waxy bundle, perpetuating the cycle of propagating asexual females (DCNR 2011).

HWA feeds on a hemlock's xylem ray parenchyma tissue by inserting a stylet into the intracellular area of the sugar storage cells at the base of the needle. The insects consume the stored starch compounds that are vital for a tree's long-term survival. The tree responds by markedly ceasing new growth and dropping its needles (McClure 2001). The actual mechanism of tree mortality is unclear -- according to Croeckling & Salom (2003), the HWA extracts too much photosynthate from the tree, which gradually kills it; others suggest that the HWA's salivary compounds are perceived as toxic to the trees causing alterations in plant structure (McClure 2001). Some suggest pest-induced embolisms in the vascular tissue cause reduced water and nutrient flow to the needles. Once infested, tree mortality often occurs within 3-4 years (Young *et al.* 1995), although some populations persist much longer (Pontius *et al.* 2006).

Tree damage resulting from HWA includes defoliation of understory trees, saplings, and seedlings, which leads to both the direct structural ecosystem changes as a result of foundation species losses, as well as indirect impacts on ecosystem processes including productivity, nutrient cycling and availability, and trophic dynamics. Death of hemlock trees opens up the canopy, increasing the light, moisture, and temperature of the forest floor. Ecosystem processes in infested stands are likely to be driven by the successional dynamics that follow hemlock mortality (Jenkins 1999). Though the effects of invaders are often viewed as a linear progression, recent models show the potential for rapid and nonlinear regime shifts in response to ecosystem change (O'Neill 2001, Carpenter 2003).

Drought causes primary and secondary physical damage as well as physiological changes in woody plants. This can weaken plants thus predisposing them to secondary invaders and pests (Taiz & Zieger 2010). Water stress triggers metabolic changes in the physiology of the plant, most notably a reduction in photosynthesis due to impaired photosynthetic capacity. It may also cause reduced growth, changing stomate function, and chlorotic needles, and belowground affects on fine roots (Taiz & Zieger 2010). All of these impacts are influenced by the severity and length of the drought, and by the vigor of the species. (Taiz & Ziegler 2010, Larcher 2003) Some studies indicate that differences in site conditions and the existence of multiple stressors, such as water stress, can accelerate a differential decline in individual tree health, increasing chances of tree mortality (Ford & Vose 2007, Orwig & Foster 1998). As water stress increases, susceptibility to disease, infections, and insect attack is heightened as the plant's ability to ward off these problems is diminished (Kozlowski *et al.* 1991). With global climate change, drought and warmer winter temperature regimes may become more common in the future of the Northeast (IPCC 2007). Several factors will promote more frequent droughts: earlier snowmelt, higher temperatures and higher variability in precipitation. Greater variability in precipitation has two implications for plant water balance: longer periods without water, and less captured in the soil following more intense storms (IPCC 2007). Hemlock is particularly susceptible to disturbance, especially water stress, due to its slow regeneration time and shallow rooting systems (Orwig & Foster 1998). Both drought and HWA infestation can reduce the carbohydrate storage in trees (Thaler & Bostoc 2004, Mattson & Haac 1987). Therefore, drought conditions may exacerbate the effects of HWA infestation (Hollensmouth 1991), relating water stress, infestation, and tree mortality. Understanding the mechanisms of drought response, survival and mortality will be critical for predicting future tree response to a changing climate (McDowell 2011).

Early symptoms of stress in forest species include reductions in photosynthetic activity and chlorophyll efficiency (Kozlowski *et al.* 1991), the principal measures in this experiment. In order to capture an interaction between drought and HWA density, the research questions addressed included:

(1) How do hemlock trees respond to drought stress?

(2) How do hemlock trees respond to differing HWA densities?
(3) How do drought stress and HWA density interact to affect tree physiology?

Our approach was to select hemlock trees with a range of HWA infestation, and impose a temporary artificial drought on half of the trees by blocking rainfall to their roots. We expected measures of photosynthesis to remain the same over time in untreated trees, and for photosynthetic efficiency to decrease over time for drought-treated trees. We also expected adelgid density to be negatively related to photosynthesis and stomatal conductance.

METHODS

Study sites included hemlock stands situated on ridges located off the Cary Pines Trail and Teahouse Road on the Cary Institute property in Millbrook, NY. Sixteen trees ranging from 18-34 cm dbh were selected for the study (Table 1). Eight of the trees were selected at random to receive a drought treatment. The "drought" was imposed on trees by skirting plastic around the trunk of the tree to prevent throughfall. Plastic skirts were created using cotton rope, notched stakes, duct tape, and 6 Mil clear plastic sheeting cut to size (20x20ft); the plastic roughly covered the projected crown area of the trees, thus presumably also covering the majority of the rooting area. The trees were located in areas that were chosen to minimize the possibility of downslope flow of water. The plastic sheeting was sealed to the trunk using polyurethane foam to divert stem flow, and was elevated above the ground using the ropes to create a tent-like structure, allowing air circulation under the plastic. Tree health was examined at the start of the study (Table 1) using a visual scaled measure of crown vigor (Table 2).

The drought treatment was imposed from July 1 to August 13. Each week after the start of the experiment, 4-5, 2-cm diameter, 10-cm deep soil cores were taken from the area under each tree. Samples were separated into organic and mineral soil in the field and composited by horizon. These samples were weighed into tins and dried at 60° C for 3 days, then reweighed to calculate soil moisture content.

%SMC = 100* (Wet weight-Dry weight)/Wet weight

Three branches were chosen per tree, and were numbered and flagged. Two random branchlet samples per branch were taken to estimate HWA density (adelgid / needle) by direct counts under a dissecting microscope. On the same branches, two replicates per branch of dark-adapted chlorophyll fluorescence were taken weekly on new growth and averaged. These measurements were taken using a chlorophyll fluorometer. The loss of light energy from the reaction center, measured as fluorescence, comes primarily from the PSII reaction (Maxwell & Johnson 2000; Taiz & Ziegler 2010). When the chloroplast or leaves have been dark-adapted, the pools of oxidation-reduction intermediates for the electron transport pathway return to an oxidized state (Maxwell & Johnson 2000). Upon illumination of a dark-adapted leaf, there is a rapid rise in fluorescent light emission from PSII followed by a decrease to steady state fluorescence as CO_2 fixation starts to occur (Maxwell & Johnson 2000). Changes in the intensity of the fluorescence emission are a sensitive reflection of changes in the photosynthetic apparatus (Maxwell & Johnson 2000). Chlorophyll fluorescence has been shown to represent changes in the health and function of the photosynthetic process (Maxwell & Johnson 2000).

Photosynthesis and stomatal conductance were measured at the end of the treatment period using a calibrated LiCor-6200 Photosynthesis System and a 1-liter chamber. Photosynthesis in understory eastern hemlocks has been reported to be light-saturated at a photon flux density of about 350 μmol m^{-2} s^{-1} (Hadley 2000), so we attempted to make measurements in sunflecks with light levels above that saturation level. Measures were taken in the field with a leaf area constant of 1 cm^2. Needle samples in the chamber were cut to size. In lab, needles were removed from the twig, needle area was measured with a LiCor Leaf Area Meter and net photosynthesis and stomatal conductance corrected for actual area.

Statistical analysis

General linear mixed effects models were used to evaluate the importance of soil moisture, and HWA density on each stress measure (CF and PS), respectively. Models were constructed to accommodate repeated measures, site and tree-level effects, as appropriate. Analyses were run using LME4 library in the statistical software R.

RESULTS

In spite of our intended drought treatment, soil moisture values increased over time in both treated and control tree plots and in both organic (Figure 1a) and mineral (Figure 1b) horizons (Time: Estimate = 3.20, SE = 0.40, $t = 7.98$, $p<0.05$). The percent soil moisture of week 6 was significantly greater than that of week 1 (pre-treatment) [$p = 2.2 \times 10^{-16}$]. These results indicate that our drought treatment did not produce the expected decline in soil moisture.

We found no difference between treatments in the slope of CF ratio over time (Estimate = 0.0003, $t = -0.74$) (Figure 2). The difference between week 7 and week 1 (pre-treatment) was also not statistically significant ($p=0.17$). Although trees were selected for treatment at random, the pre-treatment (week 1) intercepts of branch-level CF for treated trees were significantly lower on average than the measurements of the control trees ($t=-2.37$, $p<0.05$).

Because there was no statistically significant difference between the CF values over time for either control or drought treatment, we use the mean CF value across all measures from each branch. Our mixed-effects model accommodated the correlation structure among branches on a given tree, demonstrating a negative association between HWA density and mean CF Ratio at the branch scale (Figure 3).

Net photosynthesis is related to the level of light available for energy (PAR), necessitating the inclusion of differences between site and available light for each individual measurement in the model. To remove site-level differences in light availability, a model of photosynthesis (PS) dependent on log-transformed PAR values was fit and then the residuals were plotted against HWA density (Figure 4). Figure 4 suggests a tendency toward decreasing PS with increasing HWA density.

We used a similar, partial residual analysis to examine stomatal conductance after accounting for site and light differences. However, there was no significant correlation between the unexplained variance in stomatal conductance and HWA density.

DISCUSSION

While drought has the potential to cause structural and functional damage to a tree, the drought treatment attempted for this study was unsuccessful (see Figures 1a-b); the soil became wetter at every plot despite treatment. The tree skirts were sealed to the tree therefore limiting stemflow; throughfall was limited by plastic covering; and evaporation was allowed by elevation of the skirt periphery. We do not know why this treatment was not effective at reducing soil moisture, but it may have been a result of a “wick effect” (Christenson, personal communication). Hemlock forests are known for their moist soils. Creating a water deficit may have caused a gradient drawing moisture laterally from wet, surrounding soils or from deeper soils. If the experiment were to be repeated, a trench should be built around the circumference of the tree to prevent lateral capillary flow of water along this gradient. Vertical capillary flow would be difficult to stop.

Because there was no significant treatment effect on soil moisture, the lack of a treatment effect on CF ratios, PS rates and stomatal conductance are not surprising.

The lack of a significant drought treatment effect left us unable to address question 1 (*How do hemlock trees respond to drought stress?)* or question 3 *(How do drought stress and HWA density interact to affect tree physiology?)*

However, our results do shed some light on question 2 (*How do hemlock trees respond to differing HWA densities?)* As expected, we found that as HWA density increases, chlorophyll fluorescence decreases significantly. While the magnitude of response is small (from .82 to .87) this information indicates that the adelgid does impact photosynthetic efficiency, and gives a relative measure of tree photosynthetic stress due to the adelgid beyond a visual measure of vigor. Similarly, a trend suggesting a lower net photosynthesis rate as HWA density increases was found after accounting for light. More data with more range in HWA density and photosynthesis rates would be useful for future modeling of hemlock physiological response to HWA. Other studies examining the effect of the physiological stress caused by insect pests suggest similar detrimental effects on photosynthetic efficiency, the damage of herbivory directly and indirectly suppressing photosynthesis rates (Nabity *et al.* 2009; Aldea *et al*. 2007). Although few studies have characterized herbivore-induced indirect effects on photosynthesis at the leaf level, an emerging literature suggests that the loss of photosynthetic capacity following herbivory may be greater than the direct loss of photosynthetic tissues (Nabity, *et al.* 2009).

No significant relationship was found between stomatal conductance and HWA density, suggesting that the presence of the adelgid does not necessarily affect the transpiration of hemlock needles. This may indicate that the mechanism of HWA damage does not involve water transport. Again, further examination is necessary to investigate the potential impact of adelgid density on plant water balance.

CONCLUSIONS

This work confirmed that HWA infestation had a measurable physiological impact on hemlock trees. We were unable to demonstrate a drought effect or examine drought/ HWA interactions because of an unsuccessful drought treatment. Further inquiry is necessary to understand the interaction between drought and HWA density. While it is known that hemlocks respond negatively to drought stress, little is known about their response to the multiple stressors that are likely to affect them as both climate change and insect invasions continue in the future. If studied further, longer imposed and better designed methodology of drought treatment would be necessary, as would be additional measures of photosynthesis and stomatal conductance.

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APPENDIX

TABLE 1. DBH and vigor class of trees chosen for treatment (N=16).

Tree	Treatment	DBH	Vigor Class
1	Control	18.5	1
2	Drought	25	2
3	Control	28.5	1
4	Control	15	2
5	Drought	18.2	2
6	Drought	19.5	2
7	Drought	25.4	2
8	Control	21.3	2
9	Control	29.4	4
10	Drought	30.4	3
11	Control	28.6	3
12	Drought	32.4	3
13	Drought	34	3
14	Control	24	4
15	Drought	23.2	4
16	Control	28	4

TABLE 2. Crown vigor classification system.

Vigor Class	Definition
1	Tree appears healthy with <10% branch or twig mortality or foliage discoloration
2	Branch or twig mortality, or foliage discoloration on 10-25% of crown
3	Branch or twig mortality or foliage discoloration on 26-50% of crown
4	Branch or twig mortality or foliage discoloration on >50% of crown
5	No live foliage

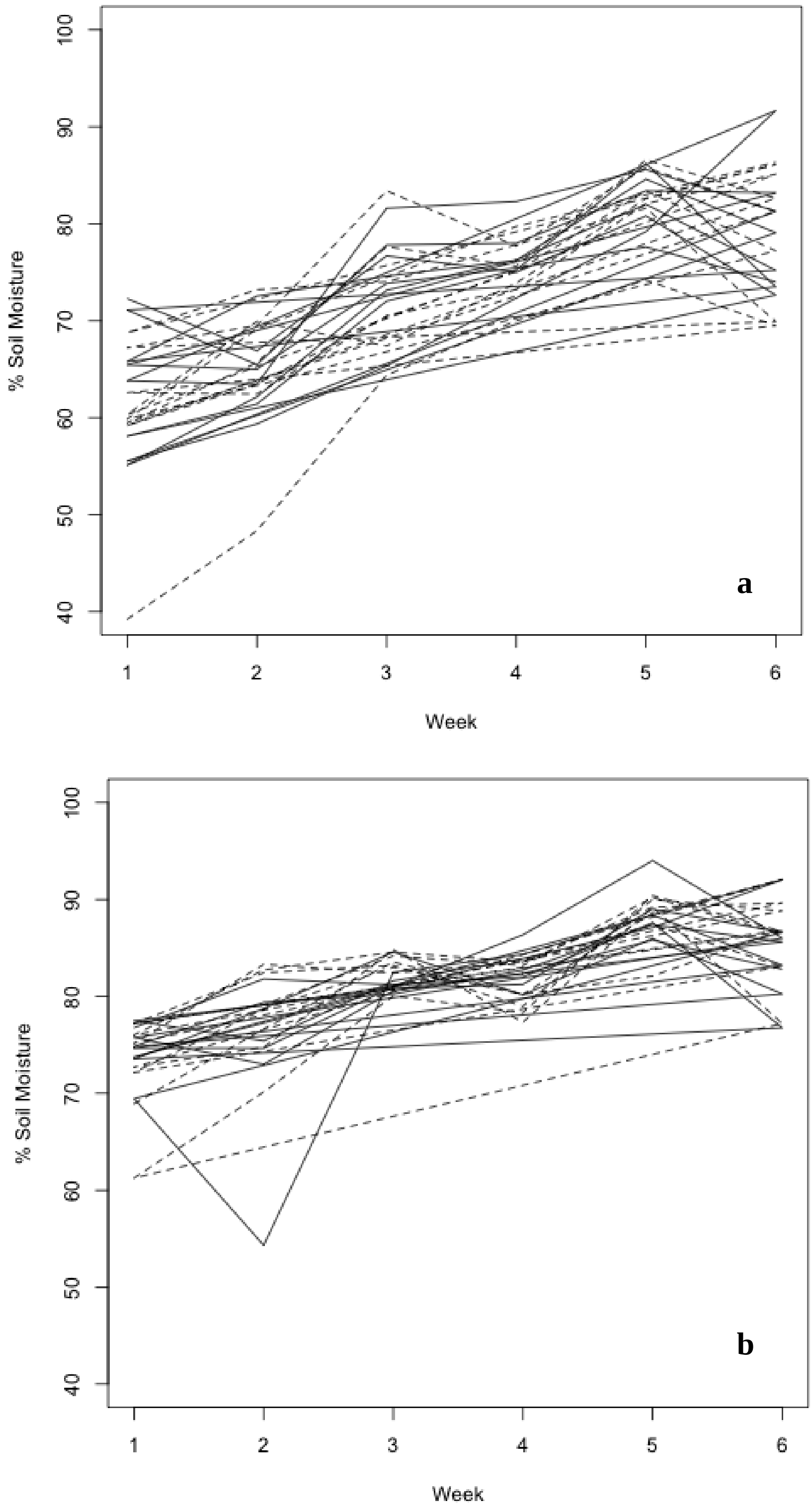

FIGURE 1a-1b. Percent soil moisture in organic (a) and mineral (b) soil. [Solid = control, Dashed = treatment].

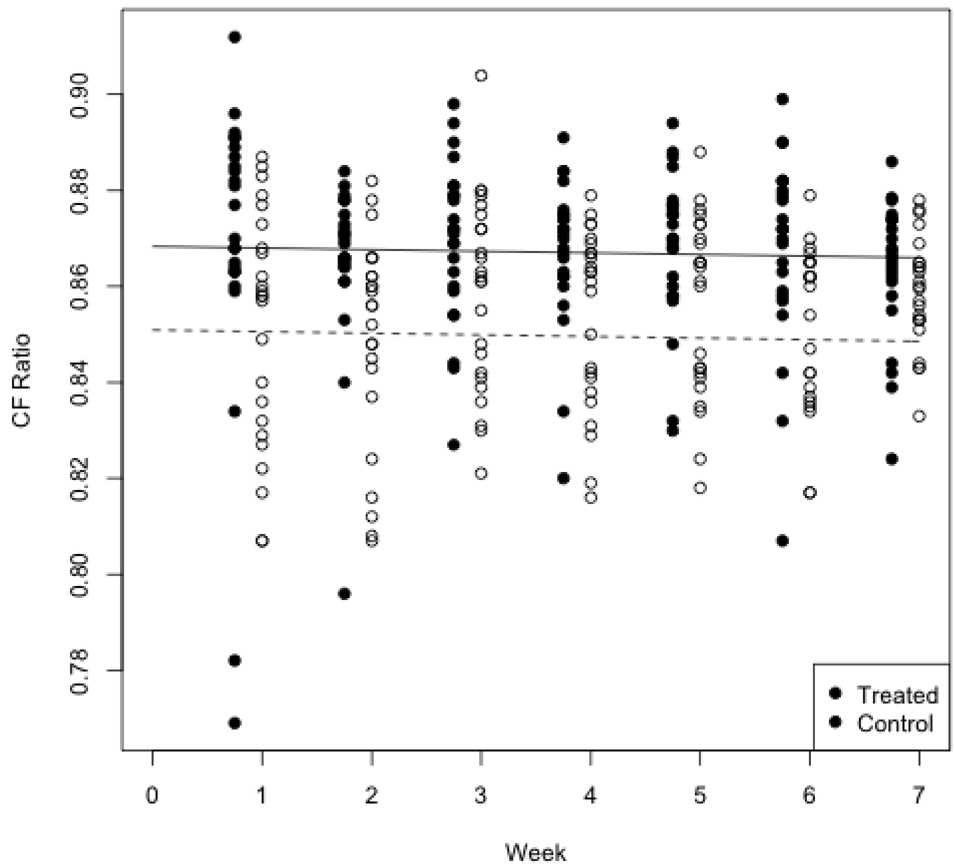

FIGURE 2. CF ratio by treatment over time. [Black = Control, Dashed = Treated].

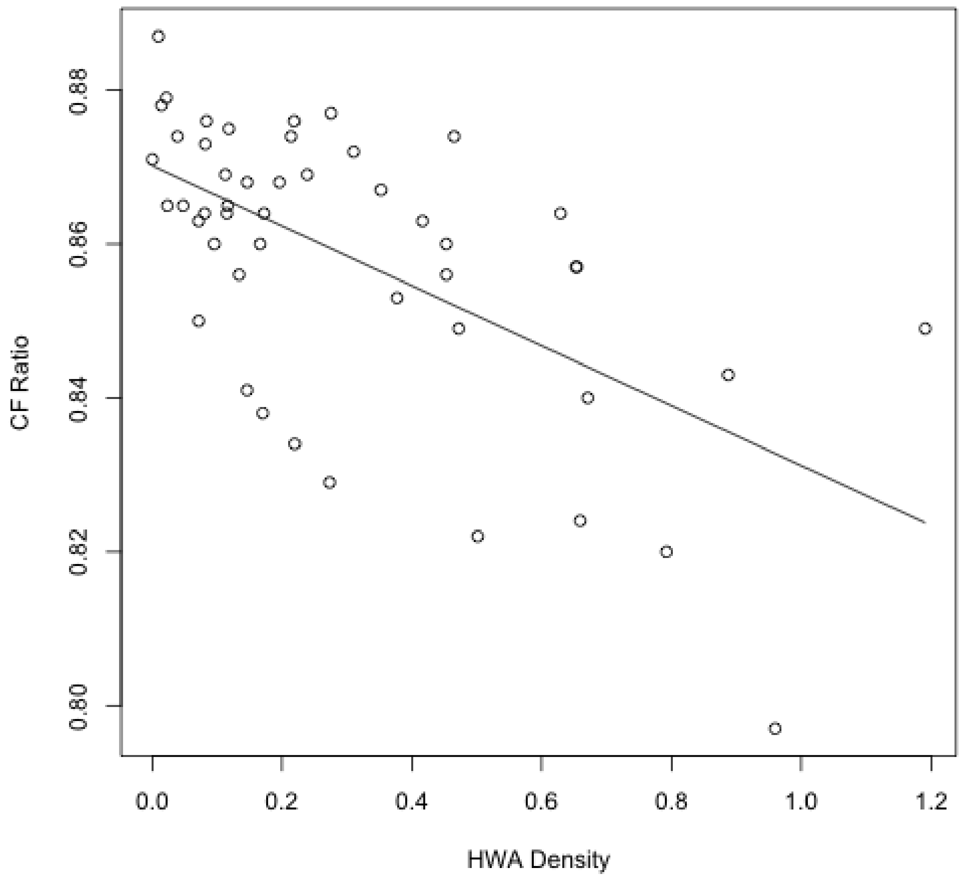

FIGURE 3. Mean CF Ratio and HWA Density. [Slope estimate = -0.039, SE= 0.01; R^2 = 0.36, p <0.001].

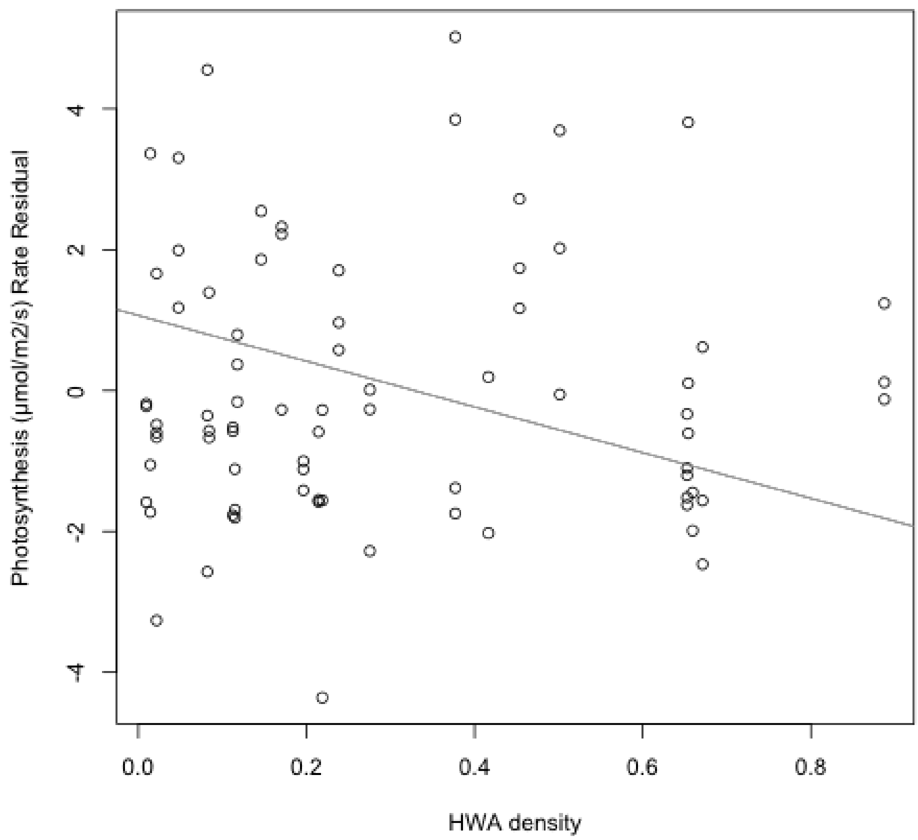

FIGURE 4. Photosynthesis residual and HWA density (Slope Estimate=-3.25, t= -2.42, p=0.10).