

ABSTRACT

MEINEKE, EMILY KATHRYN. Hot in the City: Understanding the Consequences of Urban Warming for Street Trees and their Pests (Under the direction of Dr. Steven Frank and Dr. Robert Dunn).

For over a century, scientists have known that herbivorous arthropod pests are more abundant in cities than in surrounding rural areas. However, the mechanisms for this pattern remained unknown. I investigated the effects of warming in cities, the “urban heat island effect”, on arthropod pest abundance. I predicted that urban warming would increase pest densities by inducing higher survival and reproductive rates and disrupting interactions with natural enemies. I found that urban warming increased abundance of the pest scale insect *Parthenolecanium quercifex* on willow oak *Quercus phellos* street trees by 13 times. In a greenhouse reciprocal transplant experiment, *P. quercifex* survived better in hotter conditions but only if they were from hot urban sites. This result shows that *P. quercifex* has acclimated or adapted to urban warming, one mechanism for higher *P. quercifex* abundance in urban heat islands. Further, urban heat increased *P. quercifex* fitness by disrupting interactions with parasitoid wasps. The suite of parasitoids that attacks *P. quercifex* was less effective at reducing *P. quercifex* fecundity at hot urban sites. To investigate how urban warming and arthropod pests affected urban trees, I conducted a citywide, factorial experiment across an urban warming gradient. I used pesticides to separate direct effects of warming from indirect effects of warming via higher insect pest abundance. Urban heat and higher pest abundance additively reduced tree growth, such that the direct effects of warming alone reduced citywide carbon sequestration by over 15% per year. This was in large part because hotter trees were more water stressed and, therefore, photosynthesized less than cooler trees. Using a factorial laboratory chamber experiment, I determined how these three potentially

damaging factors for trees that are increasing with climate change—warming, drought, and insect pests—affected willow oak growth and carbon storage. Results from this experiment bolstered the finding that water stress, which is exacerbated by warming, reduced urban tree carbon storage. In the absence of water stress, insect pests and warming had neutral or positive effects on trees. This novel result shows that the effects of certain insect pests will increase as trees become water stressed globally. Together, these studies provide the first evidence that urban and rural trees will grow less and, thus, store less carbon in the future as urban and global climates warm. Willow oak is one of the most resilient trees to pests and drought. Therefore, tree growth will decline even more severely in more sensitive trees and trees at the southern edges of their range.

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Hot in the City: Understanding the Consequences of Urban Warming for Street Trees and
their Pests

by
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BIOGRAPHY

I was born and raised in Greenville, North Carolina where I lived with my mother, stepfather, and 2 younger brothers before attending the University of North Carolina at Chapel Hill in 2004. I was fascinated with living things and the outdoors, which took me from the mountains of North Carolina, where I studied turtles, to Montana and Thailand where I wandered, climbed mountains, did science, and explored coral reefs. In Nevada, I joined a team as a seasonal technician to determine the success of government seeding for native grasses after fires. Subsequently, I studied restoration of longleaf pine forests, also as a seasonal technician, in South Carolina. I came back to North Carolina in 2011 to begin graduate work in Raleigh, where I have happily steeped in local nature and culture for the last 5 years. I live downtown under a big willow oak tree with my husband Joe and dog Buckley.

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CHAPTER 1: URBAN WARMING DRIVES INSECT PEST ABUNDANCE ON STREET TREES

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Urban Warming Drives Insect Pest Abundance on Street Trees

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Abstract

Cities profoundly alter biological communities, favoring some species over others, though the mechanisms that govern these changes are largely unknown. Herbivorous arthropod pests are often more abundant in urban than in rural areas, and urban outbreaks have been attributed to reduced control by predators and parasitoids and to increased susceptibility of stressed urban plants. These hypotheses, however, leave many outbreaks unexplained and fail to predict variation in pest abundance within cities. Here we show that the abundance of a common insect pest is positively related to temperature even when controlling for other habitat characteristics. The scale insect *Parthenolecanium quercifex* was 13 times more abundant on willow oak trees in the hottest parts of Raleigh, NC, in the southeastern United States, than in cooler areas, though parasitism rates were similar. We further separated the effects of heat from those of natural enemies and plant quality in a greenhouse reciprocal transplant experiment. *P. quercifex* collected from hot urban trees became more abundant in hot greenhouses than in cool greenhouses, whereas the abundance of *P. quercifex* collected from cooler urban trees remained low in hot and cool greenhouses. *Parthenolecanium quercifex* living in urban hot spots succeed with warming, and they do so because some demes have either acclimatized or adapted to high temperatures. Our results provide the first evidence that heat can be a key driver of insect pest outbreaks on urban trees. Since urban warming is similar in magnitude to global warming predicted in the next 50 years, pest abundance on city trees may foreshadow widespread outbreaks as natural forests also grow warmer.

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Introduction

Urban areas are generally hotter than surrounding rural areas [1]. This “urban heat island effect” results from the presence of less vegetation cover [2] and greater impervious surface cover [3] in cities compared to rural or natural areas [1]. Although urban warming was first noted in 1833 [4], the effects of heat on animal abundance and community characteristics in cities remain largely unknown. Instead, studies have emphasized the roles of habitat connectivity [5], [6] and resource availability [1], [7] in shaping urban animal communities. The effects of temperature deserve further attention because urban warming is becoming more extensive and more extreme as cities grow larger and is now coupled with global warming [8].

High urban temperatures should have the most pronounced effects on ectotherms, because thermal accumulation drives development in many ectothermic species [9]. Insects are of particular interest as the most diverse ectothermic taxon and because of their ecological and economic importance as pollinators [10], disease vectors [11], and plant pests [12]. Herbivorous insect pests are often more abundant in urban than in rural areas, though

the proposed mechanisms for this pattern—changes in host plant quality [13], [14] and natural enemy efficacy [15]—do not consistently explain higher herbivorous insect pest abundance [16]. We hypothesize that the urban heat island effect is the most important driver of higher insect pest abundance in cities.

To test this hypothesis, we investigated the effects of urban warming on the biology of the soft scale insect *Parthenolecanium quercifex*. As a group, scale insects are among the most important pests of forest and landscape trees and are closely related to many other pests such as aphids and whiteflies. They are also sedentary and, thus, subject to the full effects of urban warming. We therefore selected *P. quercifex*, a common scale insect pest of oaks, as a study organism to test four specific hypotheses. First, we expected urban warming to increase *P. quercifex* abundance. Our approach to testing this hypothesis differs from that of other studies because we sampled scale insects on warm and cold trees within the city rather than comparing urban to surrounding rural areas [17], [7]. Second, we hypothesized that urban warming increases *P. quercifex* abundance by decreasing parasitism. To test this hypothesis, we measured percent parasitism [18] of *P. quercifex* in hot and cold sites. Third, we tested the hypothesis that urban

warming increases *P. quercifex* abundance by increasing *P. quercifex* fecundity. This is a common physiological response to warming in ectotherms [19], [20], at least when warming pushes them toward their thermal optimum rather than beyond it [21]. Finally, we hypothesized that *P. quercifex* response to warming depends on thermal origin, such that *P. quercifex* from warmer areas have a physiological or adaptive advantage over individuals from cooler areas when placed in hot conditions. To test this hypothesis, we collected *P. quercifex* from warmer and cooler urban environments and placed them in warmer and cooler greenhouses. Because this common garden experiment provided trees with equal water and nutrients, we controlled for host plant quality, the other most common hypothesis for why herbivorous insect pests are more abundant in urban than in rural areas.

Methods

Study Organism

Soft scale insects (Hemiptera: Coccidae) are phloem-feeders on perennial plants [22]. They are commonly more abundant in cities than in rural areas [15,16]. *Parthenolecanium quercifex* is an oak pest that has one generation per year and is native to North Carolina and much of North America [22]. Adults produce eggs in the late spring, usually in May [23]. Gravid females lay a dozen to several thousand eggs in an ovisac [22]. First instars migrate from ovisacs to leaves and feed on phloem throughout summer [22], [23]. In fall they molt and migrate back to tree stems [23]. Second instars overwinter and undergo development into adults in the early spring [23].

Study Location

Raleigh has a humid subtropical climate, and the city center is located at 35.772096°N 78.638614°W. The average long-term winter temperature is 5.8°C. The average long-term summer temperature is 25.6°C. The average annual rainfall is 116.9 cm. Climate data were retrieved from the NOAA National Climatic Data Center (NCDC) (www.ncdc.noaa.gov) from the North Carolina State University weather station as 1981–2010 station normals.

Hypothesis 1) Urban Warming Increases *P. quercifex* Abundance

We used thermal maps overlaid with maps of willow oak locations in ArcMap (ArcGIS Desktop 10, Redlands, CA) to locate study sites. To create thermal maps, winter and summer temperature measurements of the study area were extracted from the 120-m thermal band (Band 6) of Landsat-5 World Reference System 2 (WRS-2) path 16, row 35 images acquired on December 12, 2005 (winter) and August 18, 2007 (summer). The summer and winter multi-spectral images were geometrically rectified by polynomial transformation with nearest-neighbor resampling to 1-meter resolution, panchromatic digital orthorectified photographs acquired in March and April 1993, archived by the North Carolina Department of Transportation. The thermal-band images were then converted from 8-bit storage values to at-satellite brightness temperature (°C). Clouds and snow were identified visually using combinations of all seven spectral bands and removed manually.

We identified 20 of the hottest (“hot”) and 20 of the coldest (“cold”) sites with at least two willow oak trees (Figure 1) in Durham, NC (1 site) and Raleigh, NC (39 sites). All sites were located in urbanized locations to minimize habitat related differences in natural enemy communities and host plant quality that might affect scale abundance. Each site was at least 200

meters away from any other site. This study was approved by the Raleigh Parks and Recreation Department, and all sites were located on public land except one site, which was located at a residence. Here, sampling was permitted by the homeowner. Sampling at all other sites was approved by the Raleigh Parks and Recreation Department.

We sampled 2nd instar scale insects by collecting terminal 30.5 cm branches from each cardinal direction of study trees in January and February 2011 using a pole pruner. In the laboratory we counted 2nd instar *P. quercifex* using a dissecting scope. We calculated mean scale insect abundance per branch on each tree. We then summed these values and divided them by the number of trees at each site (2) to generate a single insect-per-branch abundance value for each site. We compared mean scale abundance hot and cold sites with a t-test in SAS (SAS 9.1, Cary, NC).

Between April 20th and 29th, 2011, we sampled *P. quercifex* ovisacs by collecting the terminal 30.5 cm of one branch per tree at 6 hot sites and 5 cold sites (12 hot trees and 10 cold trees). To choose our study trees, we randomly selected individuals from the subset of trees occupied by 2nd instar *P. quercifex* in our first sample. We selected trees occupied by *P. quercifex* to be sure higher abundance was due to differences in population growth rather than differences in colonization between hot and cold sites. Data did not meet ANOVA assumptions, even after log transformation with $\log(x+1)$, so we compared ovisac abundance per 30.5 cm between hot and cold sites with a Kruskal-Wallis Test in SAS (SAS 9.1, Cary, NC).

Between May 20th and 25th, 2011, we sampled 1st instar scales on the same trees from which we sampled ovisacs by counting individuals on 10 leaves per study tree. We calculated mean abundance per 10 leaves on the two trees at each site. We compared $\log(x+1)$ transformed mean 1st instar abundance on 10 leaves between hot and cold sites with a t-test in SAS (SAS 9.1, Cary, NC).

To measure temperature differences between hot and cold sites, we placed ibutton thermochrons (Dallas Semiconductor of Dallas, TX) that recorded temperature 6 times per day at a subset of sites (5 hot, 6 cold). We placed thermochrons in ibutton wall mounts (Dallas Semiconductor of Dallas, TX) inside a 2.54-cm deep plastic cup to protect them from precipitation and direct sun. Thermochrons were in place from May until August 2011. We calculated daily mean and maximum temperatures in each treatment. We then compared average mean and average maximum daily temperatures at hot and cold sites using a repeated measures ANOVA in SAS (SAS 9.1, Cary, NC).

Hypothesis 2) Urban Warming Increases *P. quercifex* Abundance by Decreasing Parasitism

To test for the influence of warming on parasitoids and subsequent effects of parasitism on *P. quercifex* abundance, we collected one branch with 20 or more *P. quercifex* individuals from the same trees from which we sampled 1st instars and ovisacs on five sampling dates while the scale were developing and laying eggs (March 7, April 22, April 29, May 20, and May 27, 2011). We dissected 20 individuals per branch for parasitoid larvae and marked each individual as parasitized or not parasitized. We calculated mean percent parasitism at each site on each date. We compared mean percent parasitism between hot and cold sites using a repeated measures ANOVA in SAS (SAS 9.1, Cary, NC). To identify parasitoids that attack *P. quercifex* in Raleigh, we clipped *P. quercifex* infested branches, removed all other arthropods, and placed them in cotton-plugged vials on each date. We reared out parasitoids from March to August 2012 in an incubator

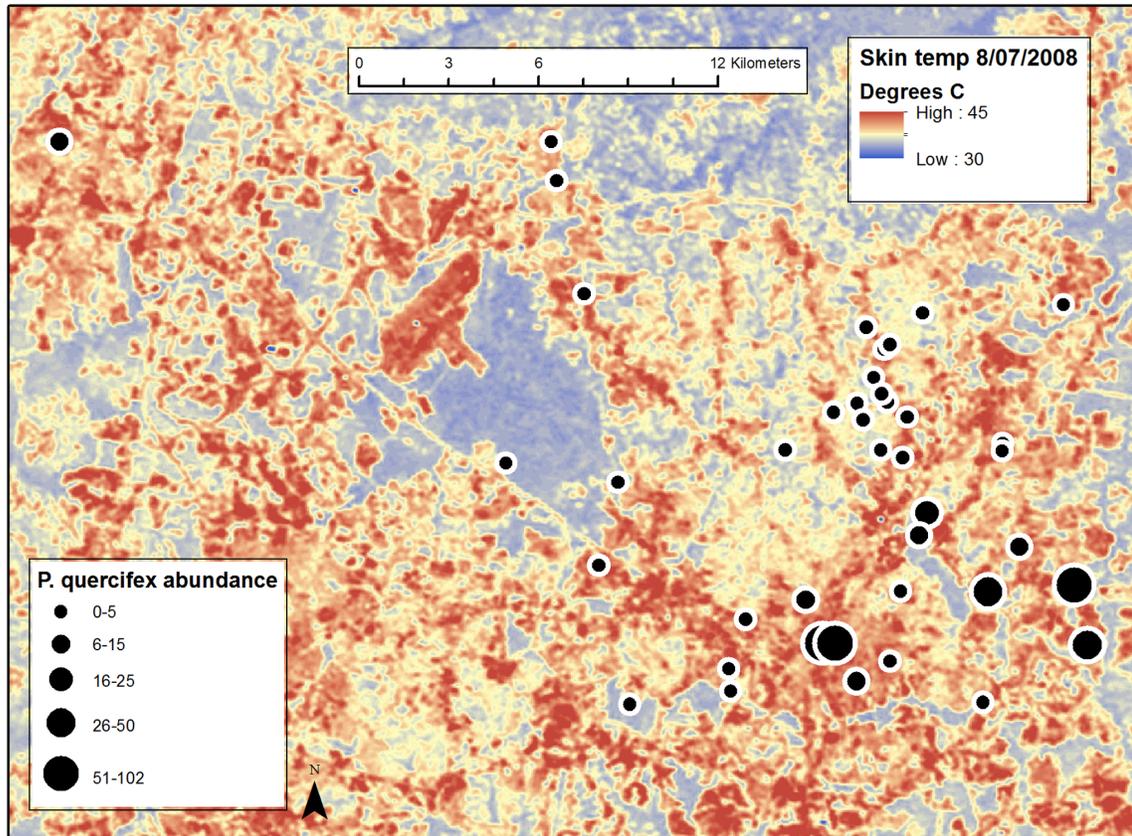


Figure 1. Thermal image overlaid with *Parthenolecanium quercifex* abundance across the Raleigh, NC urban heat island. 2nd instar *P. quercifex* abundance across the Raleigh, NC urban heat island. Dots represent relative 2nd instar *P. quercifex* abundance per 30.5 cm stem at each hot (red) and cold (blue) site (n=40) in winter 2011. The image is a thermal map of the Raleigh, NC urban heat island created from a Landsat image acquired on August 18, 2007.
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at 23°C, 50% humidity, and a 12 hr/12 hr light-dark cycle. We point-mounted each parasitoid that emerged and identified it to genus or species.

Hypothesis 3) Urban Warming Increases *P. quercifex* Abundance by Increasing *P. quercifex* Fecundity

To determine whether *P. quercifex* fecundity differed between hot and cold sites, we collected 2 ovisacs from the same trees used to assess ovisac and 1st instar abundance on April 29th, 2011. Ovisacs were returned to the laboratory in a cooler within 2 hours of collection. We emptied the eggs from each ovisac into a separate petri dish filled with 10 ml of 80% ethanol. We took a picture of each petri dish containing eggs using a Canon EOS DS126071 Rebel XT camera with a Canon EF-S 60-mm Macro lens. We used ImageJ (ImageJ 1.45 m, Bethesda, MD) to count the particles (eggs) in each image and the total area of those particles. To avoid counting multiple eggs as one, we used Image J to calculate the areas of ten eggs, found the mean of those areas, and divided the total egg area in each petri dish by the mean area of a single egg to get an egg count for each ovisac. We calculated mean egg counts for each ovisac at each site. Then we calculated mean egg count per tree

and mean egg count per site. We compared mean egg count per ovisac between hot and cold sites with a t-test in SAS (SAS 9.1, Cary, NC).

Hypothesis 4) *Parthenolecanium quercifex* Response to Warming Depends on Thermal Origin

To further isolate the effects of temperature from other biotic and abiotic effects on *P. quercifex* abundance and to test how *P. quercifex* origin affects response to temperature, we conducted a common garden experiment with a 2 by 2 factorial design, wherein we reared scales originating from hot and cold sites in hot (36°C day–18:00–6:00/32°C night–6:00–18:00) and cold (32°C day–18:00–6:00/28°C night–6:00–18:00) greenhouses. When scale matured in April 2011, we collected 4 ovisacs from a subset of our study trees (10 hot and 10 cold). We attached two ovisacs to each of 40 willow oak saplings in greenhouses at the NCSU phytotron facility in the two temperature treatments. Bare root willow oak saplings (1.04±0.02 m) were purchased from Rennerwood, Inc (Tennessee Colony, TX) and grown in 20.3 cm pots in Fafard 2P potting mix (Agawam, MA). They were fertilized 3 times per week with nutrient solution (N-P-K 10.2-1-10.7) mixed in the

NCSU phytotron (<http://www.ncsu.edu/phytotron/manual.pdf>, pp. 15–16) and watered once per day. The potting media in both treatments was kept moist to ensure that high temperature did not result in water deficiency. Two weeks before infestation, saplings were treated with Tau Fluvalinate (Mavrik, Aquaflo) 1 mL/L H₂O to ensure no other insects were being transported into the greenhouses.

After egg hatch in April 2011, we counted settled first instar nymphs on 10 leaves per tree on May 10, 17, 26, and July 15, 2011. We used repeated measures ANOVA in SAS to compare 1st instar abundance per 10 leaves among treatments.

Results

Hypothesis 1) Urban Warming Increases *P. quercifex* Abundance

We found that overwintering second instars were 13 times more abundant on hot than on cold trees ($t_{38} = 2.90$, $P = 0.006$; Figures 1 and 2A). In April 2011, ovisacs deposited by the same generation were 5.5 times more abundant on hot trees ($\chi^2_1 = 6.53$, $P = 0.011$; Figure 2C). In June 2011, the next generation of 1st instars was over 7 times more abundant on hot than cold trees ($t_9 = 2.46$, $P = 0.043$; Figure 2B).

There was a significant interaction between site temperature and time, wherein the extent of the differences in mean average temperatures ($F_{112, 1120} = 1.96$, $P < 0.0001$) between hot and cold sites depended on time of year. Similarly, the interaction between site temperature and time was marginally significant for mean maximum temperatures ($F_{112, 1120} = 1.23$, $P = 0.0583$). Mean average hot site temperatures were between 0–2.4°C higher than mean average temperature at cold site temperatures ($F_{1, 10} = 7.90$, $P = 0.0185$; Figure 3A), and mean maximum daily temperatures at hot sites were between 0–3.8°C warmer than mean maximum daily temperatures at cold sites ($F_{1, 10} = 6.42$, $P = 0.0297$; Figure 3B).

Hypothesis 2) Urban Warming Increases *P. quercifex* Abundance by Decreasing Parasitism

We reared six parasitoid species from *P. quercifex*: *Coccophagus lycimnia* Walker (Hymenoptera: Aphelinidae), *Pachyneuron altiscutum* Howard (Hymenoptera: Pteromalidae), *Eumotus lividus* Ashmead (Hymenoptera: Pteromalidae), *Encyrtus fuscus* Howard (Hymenoptera: Encyrtidae), *Blastothrix* sp. Mayr (Hymenoptera: Encyrtidae), and *Metaphycus* sp. Mercet (Hymenoptera: Encyrtidae). Percent parasitism did not differ between *P. quercifex* from hot and cold sites ($F_{1, 6.45} = 0.21$, $P = 0.6631$; Figure 4).

Hypothesis 3) Urban Warming Increases *P. quercifex* Abundance by Increasing *P. quercifex* Fecundity

The number of eggs in ovisacs from hot and cold sites did not differ ($t_9 = 1.87$, $P = 0.094$).

Hypothesis 4) *P. quercifex* Response to Warming Depends on Thermal Origin

The effect of greenhouse temperature on scale abundance depended on scale origin, such that *P. quercifex* collected from hot trees reared in hot greenhouses were over twice as abundant as *P. quercifex* in any other treatment ($F_{1, 134} = 11.57$, p -value = 0.0009; Table 1, Figure 5). *P. quercifex* from cold trees did not become more abundant when reared in hot greenhouses. In the cold greenhouse, *P. quercifex* from hot trees were significantly more abundant than *P. quercifex* from cold trees; still, they were less than half as abundant as in hot greenhouses.

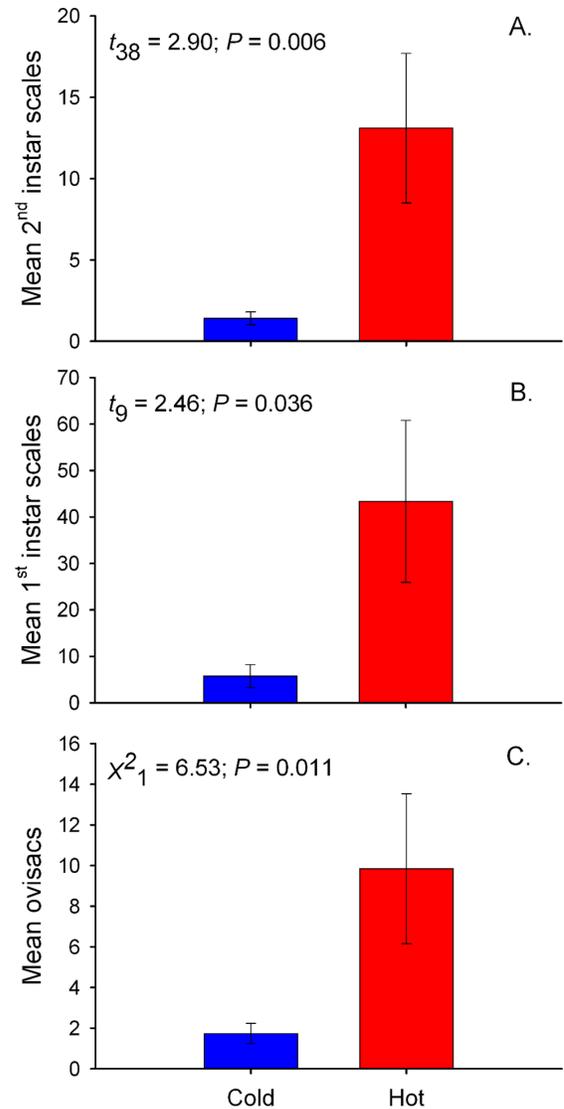


Figure 2. *Parthenolecanium quercifex* abundance across the Raleigh, NC urban heat island. Abundance of *P. quercifex* on hot and cold urban trees. Bars represent the mean (\pm SEM) abundance of (A) 2nd instars in winter 2011 ($n = 40$); (B) 1st instars in June 2011 ($n = 11$); and (C) ovisacs in spring 2011 ($n = 11$) on 30.5-cm terminal branches of hot (red) and cold (blue) urban trees in Raleigh, NC. doi:10.1371/journal.pone.0059687.g002

Discussion

We found urban warming directly leads to higher *P. quercifex* abundance. While the two most common hypotheses for elevated pest abundance in cities are changes in host plant quality and natural enemy efficacy [16], we found no evidence that either of these factors contribute to *P. quercifex* abundance patterns across the Raleigh, NC urban heat island. We also found no evidence that urban warming directly affects *P. quercifex* fecundity. Instead, we found evidence that *P. quercifex* populations may be locally

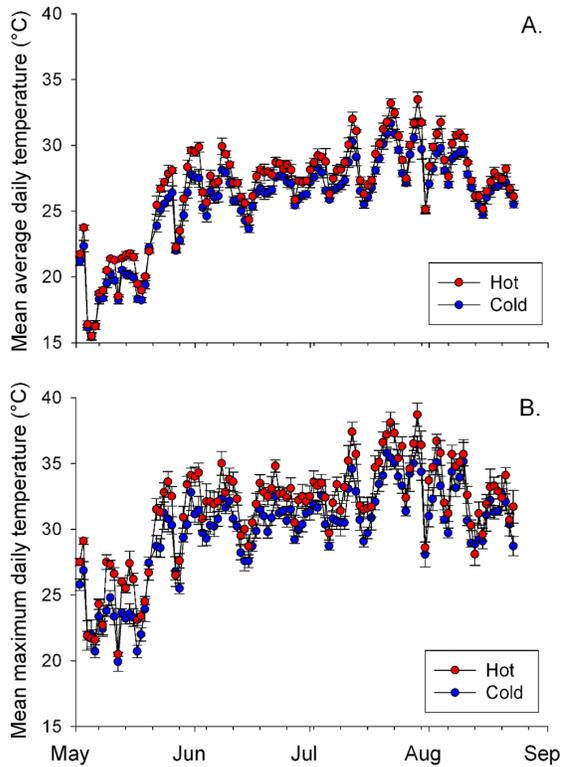


Figure 3. Average and maximum temperature differences between hot and cold sites. Temperatures recorded on ibuttons at 'hot' and 'cold' sites in Raleigh, NC May 2, 2011- August 23, 2011. Dots represent mean (\pm SEM) a) average daily temperature ($^{\circ}$ C) and b) mean maximum daily temperature at hot and cold sites. Average daily mean temperatures were significantly higher at hot sites ($F_{1, 10}=7.90$, $P=0.0185$), as were mean daily maximum temperatures ($F_{1, 10}=6.42$, $P=0.0297$). The extent of the difference between average ($F_{112, 1120}=1.96$, $P<0.0001$) and maximum daily temperatures ($F_{112, 1120}=1.23$, $P=0.0583$) depended on time of year. doi:10.1371/journal.pone.0059687.g003

adapted, or individuals acclimatized, to the temperature of the urban habitat patches in which they reside.

Urban trees are frequently stressed due to lack of water and nutrients [24], [25]. In some cases, stress can reduce tree defenses, leading to higher herbivore abundance [26]. Because our study sites were all in urban habitats, we have no reason to believe that nutrient levels available to trees covaried with temperature. It is conceivable that warm trees are more water stressed, and such a possibility deserves study. However, water stress tends to lead to decreases in the abundance of piercing-sucking herbivores [27], [28], which suggests that water stress should lead to lower *P. quercifex* abundance in hot urban areas. We observe the opposite pattern. Additionally, in our common garden experiment, we watered trees daily and provided equal nutrients to all trees to minimize any effects of water or nutrient stress. It is unlikely that differences in tree stress or quality account for the difference in scale abundance between hot and cold sites.

Natural enemies are often less abundant and diverse in urban than rural habitats. This difference has been cited to explain higher pest abundance in cities [16], [15]. All our study sites were within urban habitats, so—given that natural enemies tend to be

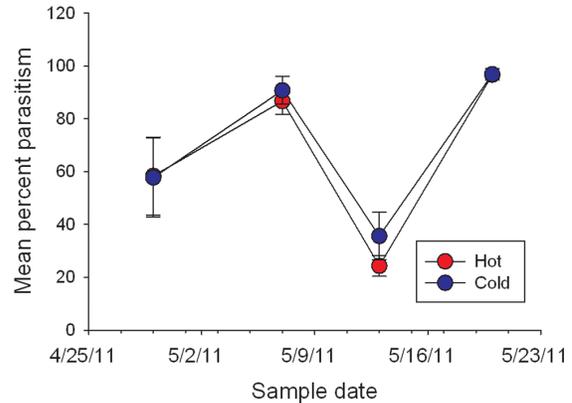


Figure 4. Percent parasitism of *P. quercifex* on hot and cold urban trees. Bars represent the mean (\pm SEM) percent of dissected 2nd instars, adults, and ovisacs that had been parasitized on hot (red) and cold (blue) urban trees in Raleigh, NC on four dates in 2011. Temperature treatment had no significant effect on percent parasitism ($F_{1, 6.45}=0.21$, $P=0.6631$, $n=11$). doi:10.1371/journal.pone.0059687.g004

relatively good dispersers [29], [30]—natural enemy communities should be similar among trees. As such, it is not surprising that we did not find a difference in percent parasitism between *P. quercifex* from hot and cold sites. Differences in parasitoid efficacy do not account for greater *P. quercifex* abundance on hot trees, as percent parasitism of *P. quercifex* on hot trees was equal to that of cold trees. Additionally, *P. quercifex* was more abundant in hot chambers in our greenhouse experiment, which excluded natural enemies. Thus, reduction of biological control by parasitoids does not explain high scale abundance at hot sites.

Our common garden experiment shows that *P. quercifex* is locally acclimated or adapted to urban thermal conditions and that this directly leads to higher abundance. *P. quercifex* from hot urban areas became almost 4 times more abundant than those from cold urban areas when placed in hot greenhouses. This effect is likely due to differences in survival, because we found no differences in fecundity between *P. quercifex* from hot and cold sites. We suggest that *P. quercifex* may locally adapt in response to urban warming, as other studies provide evidence for local adaptation in scale insects [31], [32]. The scale insect life cycle, which is often parthenogenetic and highly localized, inhibits gene flow [33], and evidence suggests this could lead to differentiation at small spatial scales

Table 1. Statistics for repeated measures ANOVA of *P. quercifex* abundance in common garden experiment. (An * denotes an interaction.)

Effect	Ndf, Ddf	F	P
Date	3, 134	0.35	0.7867
Source temp.	1, 134	46.57	<0.0001
Date* Source temp.	3, 134	0.04	0.9891
Greenhouse	1, 134	31.65	<0.0001
Date* Greenhouse	3, 134	0.67	0.5698
Source temp.* Greenhouse	1, 134	11.57	0.0009
Date* Source temp.* Greenhouse	3, 134	0.01	0.9987

doi:10.1371/journal.pone.0059687.t001

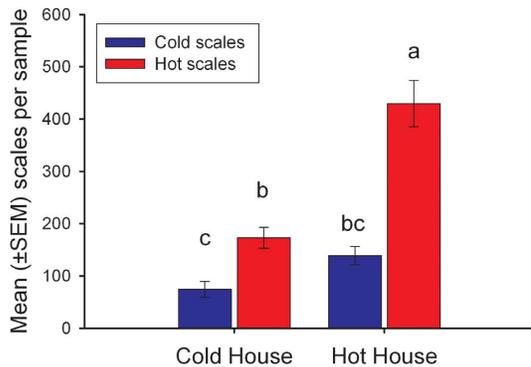


Figure 5. Abundance of *P. quercifex* in a common garden experiment. Bars represent the mean (\pm SEM) *P. quercifex* 1st instars per 10 leaves. We calculated mean abundance across 4 sample dates (May 10, 17, 26, and July 15, 2011) since Repeated Measures ANOVA indicated there was no interaction of time with ovicase source or temperature treatment. 1st instars hatched from ovicases collected on hot (red) and cold (blue) urban trees, then reared on 'hot' and 'cold' greenhouse saplings (x axis). There was a significant interaction between *P. quercifex* ovicase source temperature and greenhouse temperature ($F_{1, 134} = 11.57$, $P = 0.0009$, $n = 40$). Detailed statistics are available in Table 1.
doi:10.1371/journal.pone.0059687.g005

[34]. Further, at least one other scale insects species has been shown to adapt to thermal conditions within its introduced range [35]. However, we cannot eliminate the possibility that observed abundance patterns resulted from maternal effects [36] or phenotypic plasticity of offspring leading to acclimation [37], rather than from genetic differences between *P. quercifex* from hotter and colder areas [38]. While the specific mechanism by

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which warming increases *P. quercifex* abundance warrants further investigation, our findings show that *P. quercifex* are primed to survive better in response to warming, be it urban or global.

For more than a century, scientists have documented that arthropod pests, including scale insects [39], are more abundant on urban trees than rural trees [16]. We provide evidence that urban heat may explain this effect, and we show that small temperature differences predict changes of an order of magnitude in pest abundance. We observed this effect over a temperature gradient common in many urban heat islands [1], indicating that urban warming poses a broad and immediate threat to urban trees and the services they provide, including cooling and carbon sequestration [2]. The adaptation or acclimation of herbivorous pests to warm environments may represent an ecological tipping point after which arthropod pests can overwhelm plant defenses and escape natural enemy control. Furthermore, temperature increases of similar magnitude are predicted under global climate change [40]. If rising global temperatures trigger an herbivore response similar to the one we observed in the city, then both urban and rural trees may be threatened by greatly increased herbivory in the future.

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Author Contributions

Conceived and designed the experiments: EKM RRD SDF. Performed the experiments: EKM SDF. Analyzed the data: EKM SDF JOS. Contributed reagents/materials/analysis tools: EKM SDF JOS RRD. Wrote the paper: EKM SDF JOS RRD.

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CHAPTER 2: EARLY PEST DEVELOPMENT AND LOSS OF BIOLOGICAL CONTROL ARE ASSOCIATED WITH URBAN WARMING

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Global change biology

Early pest development and loss of biological control are associated with urban warming

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Climate warming is predicted to cause many changes in ectotherm communities, one of which is phenological mismatch, wherein one species' development advances relative to an associated species or community. Phenological mismatches already lead to loss of pollination services, and we predict that they also cause loss of biological control. Here, we provide evidence that a pest develops earlier due to urban warming but that phenology of its parasitoid community does not similarly advance. This mismatch is associated with greater egg production that likely leads to more pests on trees.

1. Introduction

Climate warming can cause phenological mismatches between associated species. These mismatches can disrupt trophic interactions and reduce ecosystem services [1]. For example, when flowers open before bees become active, pollination is reduced, and plants produce fewer seeds [2]. Warming could also reduce biological control of pests if pest phenology advances more than predator or parasitoid phenology. If mismatches between predators and prey occur anywhere, it is probably in cities where air temperatures are up to 12°C hotter than in surrounding areas [3]. Empirically, herbivorous insect pests are often more abundant on urban than rural plants. In part, this is because warming increases their survival, fecundity and population growth rate relative to individuals in cooler urban areas [4,5]. However, high pest abundance in cities could also result from poor biological control [6] due to phenological mismatches between pests and natural enemies.

In a previous study, we found that the scale insect *Parthenolecanium quercifex* (Hemiptera: Coccidae) is 12 times more abundant on trees in the warmest urban habitats [4]. Female *P. quercifex* are attacked by several hymenopteran parasitoids that kill them or reduce their fecundity. In this study, we monitored *P. quercifex* and parasitoid phenology in hot urban sites and in similar urban sites that were cooler, relative to the hot sites. We tested if warming reduced parasitism rate or changed parasitoid reduction of *P. quercifex* egg number.

2. Material and methods

(a) Study organisms

Parthenolecanium quercifex is a common, native, univoltine pest on urban oak trees. In North Carolina, USA, adults produce eggs for about three weeks between April and May on willow oak (*Quercus phellos*). Females die after depositing eggs underneath their body cavities. Eggs hatch before June.

Parasitoids develop in *P. quercifex* throughout its life cycle [7]. Our study focused on species that are within *P. quercifex* during oviposition in April and May. Although the specifics of the parasitoids' life cycles are not known, adults are absent in early spring and become more abundant throughout the season, which suggests parasitoids

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overwinter in *P. quercifex* as larvae. To confirm identities of *P. quercifex* parasitoids in our study area, Raleigh, NC, USA, we collected 20 *P. quercifex* from each of 16 trees across the city on 16 April 2012 and placed them in individual 1.5 ml vials with cotton plugs, in an incubator (25°C and 70% RH) until eclosion. We identified parasitoids to genus and deposited voucher specimens at the North Carolina State University Insect Museum.

(b) Study system

Previously, we studied *P. quercifex* abundance on willow oaks at 40 sites in Raleigh [4]. We overlaid a surface temperature layer with a tree inventory in ArcGIS (ESRI 2011) to choose study sites. We selected 20 sites from the hottest zones on the map and 20 sites from the coolest zones with two adjacent willow oak trees. For this study, we chose eight hot and eight cold sites that had detectable numbers of *P. quercifex* from our original 40 sites. One of our hot sites was removed from the study due to construction that interrupted data collection. Although other variables, such as pollution, water availability, and pesticide and fertilizer runoff could have contributed to host and parasitoid abundance and phenology, we assumed that temperature was the dominant effect. As discussed below, including site as a random effect in our models at least partially accounted for these variables. We placed an ibutton thermachron (model DS1921G, precision: 0.5°C, accuracy: $\pm 1.0^\circ\text{C}$; Dallas Semiconductor, TX, USA) on an outer branch of one tree at each site from February to May 2014 to confirm that air temperatures at hot sites were higher than those at cold sites. The ibuttons recorded temperature every hour. Ibuttons were lost at five sites and malfunctioned at three sites. We present temperature data from five cold and three hot sites. To compare average temperatures at hot and cold sites, we used a linear mixed effects model in the nlme package R [8] with temperature category and date as factors and site as a random effect. We compared autoregressive, unstructured and compound symmetry correlation structures, and chose the autoregressive structure based on Akaike's Information Criterion (AIC). Hereafter, models of this type were considered significantly different when ΔAIC was more than 2. Then we compared models with all predictor combinations, including interaction terms, using AICc. We used this method for all linear mixed effects model selections.

(i) Prediction 1: *Parthenolecanium quercifex* develops earlier in warmer urban areas but parasitoids do not

On 2 and 18 March, 1 and 16 April and 1 May, we collected between 10 and 20 *P. quercifex* from two adjacent trees at each of our 16 study sites. We categorized each *P. quercifex* as second instar, adult, adult with eggs, or dead adult with eggs and dissected each to look for parasitoid larvae. We tested our prediction with a generalized linear mixed model in the GLIMMIX Procedure of SAS (SAS Institute 2013). Date, categorical temperature and their interaction were fixed effects, and 'site' was a random effect. To determine how temperature effects percent parasitism and parasitoid larval phenology, we used logit-transformed ($\log((x+1)/(1-x+1))$) proportion of scales with parasitoid larvae as a response, temperature and date as predictors, and site as a random effect in a linear mixed effects model.

We placed one 7.6×12.7 cm yellow sticky card (Olson Products, OH, USA) in both trees at each site, each time we collected *P. quercifex* life stage data to determine parasitoid flight phenology at hot and cold sites. We collected cards on the next date and counted and identified *P. quercifex* parasitoids. We used $\log(x+1)$ -transformed parasitoid count on sticky cards as a response, temperature and date as predictors, and site as a random effect in a linear mixed effects model to determine how temperature affected parasitoid abundance and parasitoid flight phenology.

(ii) Prediction 2: Parasitoids reduce *Parthenolecanium quercifex* egg count more in cold than in hot urban areas

We harvested *P. quercifex* egg sacs from sites used in Prediction 1 along with three additional hot sites and two additional cold sites from our original study [4] to add replicates, for a total of 10 hot and 10 cold sites. At each site, we collected 40 dead females on 23–24 April 2012, before *P. quercifex* eggs hatched. Females and their eggs were inspected for live parasitoids and parasitoid exit holes and were placed into vials with 95% ethanol. We put eggs from each sample into a Petri dish with 10 ml of 95% ethanol and photographed them using a Canon EOS 7D camera with a Canon EF-S compact 50 mm macro lens. We used ImageJ to count area occupied by eggs in each photo and calculated the area of 10 eggs in five random Petri dishes from each site to get average egg size per site. We divided egg area per dish by average egg size to calculate egg count.

Different parasitoid communities attack *P. quercifex* during each life stage. We focused on parasitoid species that are within *P. quercifex* during oviposition. We separated the dataset into two categories: *P. quercifex* with eggs and *P. quercifex* without eggs. With those that produced eggs, we tested for the effects of warming and parasitoid presence on egg count with a generalized linear mixed model in the GLIMMIX Procedure of SAS. Parasitoids and temperature were fixed effects, and site was a random effect. We assumed that the data were drawn from a gamma distribution, and the log of the expected mean response was a function of the fixed effects.

3. Results

A discussion of parasitoid identities and communities is in the electronic supplementary material, figure S1a,b.

(a) Temperature

Sites designated as 'hot' were significantly warmer than 'cold' sites (mean \pm s.e.m. $19.0 \pm 0.5^\circ\text{C}$ and $18.5 \pm 0.5^\circ\text{C}$, respectively; $F_{1,8} = 5.51$, $p = 0.0469$; electronic supplementary material, figure S2a,b), depending on day ($\Delta\text{AICc} = 296.72$; assigned temp. \times date: $F_{103,748} = 2.44$, $p < 0.0001$) wherein the hot and cold sites were more different earlier in the year than later.

(i) Prediction 1: *Parthenolecanium quercifex* develops earlier in warmer urban areas but parasitoids do not

The effects of temperature on the proportion of *P. quercifex* producing eggs depended on date (GLIMMIX model; $F_{3,45} = 3.91$, $p = 0.0146$), such that a higher proportion of *P. quercifex* at hot sites produced eggs on early dates (figure 1a). This indicates that *P. quercifex* produces eggs earlier at hot sites, supporting our prediction that warming advances *P. quercifex* phenology.

Date was the only significant factor predicting the proportion of *P. quercifex* with parasitoid larvae (figure 2a; $\Delta\text{AICc} = 2.36$; $F_{1,42} = 98.52$, $p < 0.0001$), indicating that parasitoid larvae did not appear earlier at hot sites and that percent parasitism was not different between hot and cold sites. As might be expected given the higher density of *P. quercifex* at hot sites, adult parasitoid abundance was also higher at hot sites (figure 1b; $F_{1,13} = 9.24$, $p < 0.0095$) and significantly increased as the season progressed ($F_{1,53} = 174.55$, $p < 0.0001$), but the best model did not include an interaction between temperature and time ($\Delta\text{AICc} = 3.35$). Collectively, our data show that parasitoid abundance increased with *P. quercifex* abundance, but that percent

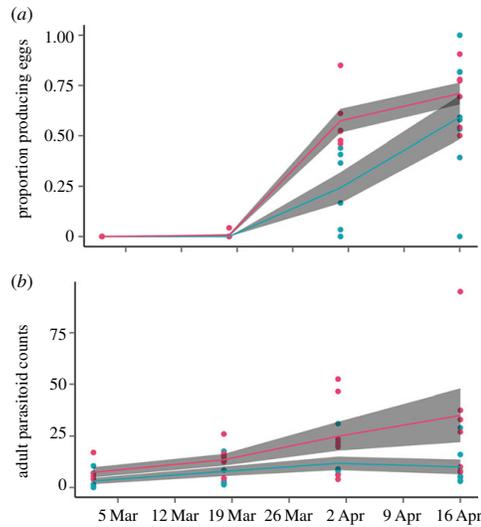


Figure 1. (a) *Parthenolecanium quercifex* produces eggs early in warmer parts of the city, (b) but emergence timing of adult parasitoids is not affected. (Hot sites, pink; cold sites, blue.)

parasitism and parasitoid phenology were not significantly different at hot and cold sites.

(ii) Prediction 2: Parasitoids reduce *Parthenolecanium quercifex* egg count more in cold than in hot urban areas

Temperature and parasitism interacted to affect egg count (figure 2b; $F_{1,589} = 3.73$, $p = 0.0258$), as parasitized *P. quercifex* that oviposited produced twice as many eggs at hot sites than at cold sites. Overall, egg numbers were not different between hot and cold sites ($F_{1,17} = 1.96$, $p = 0.1799$).

4. Discussion

Ecologists have predicted for several decades that insect pests will become more abundant as the earth warms [9]. We show for the first time that pests and their parasitoids in the city undergo phenological mismatches akin to those predicted to occur due to global warming [10]. The scale insect *P. quercifex* oviposited earlier in warmer urban areas, relative to similar, cooler areas, whereas the phenology of its parasitoid community did not similarly advance. This mismatch between parasitoid and host phenology did not reduce the proportion of *P. quercifex* scale insects that were parasitized. However, parasitized *P. quercifex* on trees in hot zones produced twice as many eggs as parasitized individuals on cooler urban trees, while the number of eggs produced by unparasitized individuals did not differ with temperature, as found in previous studies of other scale insect species [5]. Overall, we provide evidence that phenological mismatches between scale insects and their parasitoids could lead to more pests on street trees.

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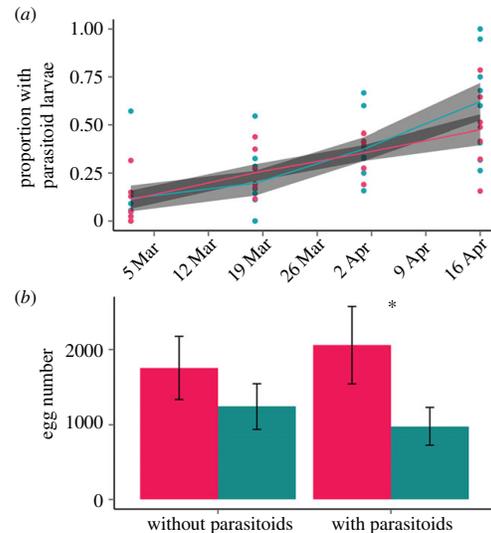


Figure 2. (a) The same proportion of *P. quercifex* are parasitized across urban temperatures, but (b) *Parthenolecanium quercifex* in hot urban zones produce twice as many eggs when parasitized (hot sites, pink; cold sites, blue; asterisk indicates significant difference within the parasitoid treatment).

At least two factors may contribute to the phenological mismatches that we observe. First, *P. quercifex* are mostly sedentary, and so have no means to escape or moderate high temperatures. By contrast, most natural enemies can move at greater spatial scales and through hot and cold zones within the city. Even at small spatial scales, mobile natural enemies like parasitoids can buffer high temperatures by moving to cooler parts of their habitats [11]. In our system, this may mean moving deeper into the canopy or within bark crevices. Additionally, endoparasitoids may be buffered from extreme temperatures, because they develop within their hosts [12].

This study documents a new mechanism for urban pest outbreaks, which damage trees and reduce ecosystem services [13]. Warming may increase pest abundance in many habitats through multiple mechanisms, including phenological mismatches that reduce biological control.

Disclaimer. EPA has not officially endorsed this publication and the views expressed herein may not reflect the views of the EPA.

Data accessibility. Data will be deposited in the NCEAS repository.

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CHAPTER 3: URBAN WARMING REDUCES ABOVEGROUND CARBON STORAGE

Abstract

A substantial amount of global carbon is stored in mature trees. However, no experiments to date test how warming affects mature tree carbon storage. Using a unique, citywide, factorial experiment, I investigated how warming and insect herbivory affected physiological function and carbon sequestration (carbon stored per year) of mature trees. Urban warming increased herbivorous arthropod abundance on trees, but these herbivores had negligible effects on tree carbon sequestration. Instead, warming directly reduced citywide carbon sequestration by over 15% per year, in part by reducing photosynthesis at hotter sites. Ecosystem service assessments that do not consider urban climate may dramatically overestimate urban tree carbon storage. Because urban and global warming are becoming more intense, urban trees will sequester even less carbon in the future.

Introduction

Trees are critical components of nearly every ecosystem on earth. They regulate local [1] and global climates [2-4], filter air [5] and water [6], and provide habitats for a large proportion of known biodiversity [7]. One of the most important services trees provide is carbon storage. During photosynthesis, trees take up CO₂ from the atmosphere and incorporate its components into their tissues. In doing so, they reduce CO₂ concentrations in the atmosphere and future climate warming. Despite considerable research on how climate change will affect trees and the services they provide [8-10], predictions remain contradictory, in part because experiments on mature trees are rare. Further, no experiments have tested how mature trees respond to warming, a key component of climate change.

Net effects of future warming on temperate trees remain uncertain. Theoretically, warming could directly reduce tree growth and, thus, carbon storage by intensifying water stress [11], reducing photosynthesis, or both [12]. Warming could increase growth by extending growing seasons [13]. Warming could also indirectly decrease tree growth by increasing herbivorous arthropod abundances [14]. Outbreaks of herbivorous pests, such as the pine processionary moth [15] and the mountain pine beetle [16], have become more frequent due to climate change, sometimes turning forests from carbon sinks into carbon sources by killing trees [17]. However, chronic herbivory—sub-lethal feeding by native arthropods throughout trees' lives, typically in low abundances—has rarely been studied. This is despite the fact that most tree species worldwide have chronic herbivores such as

many scale insect and aphid species, several of which are already more abundant due to warming [18,19].

Urban forests provide a unique opportunity to determine the effects of recent, rapid warming on mature trees. In many cities, the urban heat island effect, or “urban warming,” is comparable in magnitude to warming expected globally in the next century [23]. Urban warming is caused by hard surfaces that absorb radiation from the sun and emit it as heat [20]. Because these hard surfaces are patchily distributed, cities are thermal mosaics [21,22]. These mosaics provide ready-made experiments, in which trees have been planted and then subjected to a variety of thermal environments. Further, trees in the hottest parts of cities are often infested with chronic herbivores that are rare in rural forests and cooler parts of cities, but become pests due to urban warming. For example, urban warming increases densities of two native scale insect species that survive better [24] and produce more eggs [25,26] at hotter urban sites.

To determine how warming affects tree carbon storage, I conducted a three-year, factorial experiment across a range of urban warming intensities. Across this temperature range, I treated some trees with horticultural oil to reduce chronic herbivore abundance, separating direct effects of warming on tree physiology from indirect effects through herbivores. First, I measured how warming affected tree growth. To identify the mechanisms by which warming affected tree growth, I measured leaf gas exchange and water stress. This study is the first to use urban habitats to measure how warming affects function of mature trees and to scale these effects up to an entire city.

Methods

Study system

The focal tree species in this study, willow oak *Quercus phellos*, is one of the largest, most commonly planted shade tree species in the eastern US. Its most common arthropod pest species is the oak lecanium scale insect *Parthenolecanium quercifex*. *P. quercifex* is univoltine. In spring, females feed on phloem sap from branches and produce up to 3000 eggs per female (Meineke et al., unpublished data). Newly hatched nymphs move to leaves to feed during summer and return to branches before leaf fall to overwinter. In general, scale insects can reduce tree growth by feeding on sap and by promoting sooty mold on leaves, which blocks leaf light interception and photosynthesis [27].

Study design

Raleigh, NC has a humid, subtropical climate and is located at 35.77°N 78.64°W. As in Youngsteadt et al. 2015, I used a Landsat infrared layer of surface temperature in April 7, 2011 to choose study sites. I overlaid a map of Raleigh street trees provided by the Parks, Recreation, and Cultural Resources Department with the thermal image and chose 10 relatively hot sites (above median surface temperature of all street trees) and 10 relatively cool sites, each of which contained 2 willow oak trees less than 30 m apart. The mean (\pm SEM) study tree diameter at breast height (DBH) was 20.9 \pm 1.2 cm.

To remove scale insects and other small, soft-bodied chronic herbivores, I used a mist blower (model: 451, Solo, Newport News, VA, USA) to treat one tree per site with

horticultural oil (SuffOil-X, BioWorks, Victor, NY, USA) on 6 dates (March, July and September 2013; February, March, April, and May 2014). I applied the oil until leaves were wet, using a concentration of 2 L per 100 L of water in March 2013, September 2013, February 2014, March 2014, and 1 L per 100 L of water in July 2013, April 2014, and May 2014. These treatments created a split plot design with temperature as the whole plot factor and oil as the split plot factor.

I installed one ibutton thermocron data logger (model: DS1921G, precision: 0.5 °C, accuracy: ± 1.0 °C, Dallas Semiconductor, TX, USA) on the northern, bottommost branch of one tree per site. I placed ibuttons below branches to reduce sun exposure associated with malfunctions in other studies [25]. Ibuttons were housed in wall mounts (Dallas Semiconductor of Dallas, TX) inside 2.54-cm deep plastic cups to protect them from precipitation and direct sun.

At the study site Raleigh, NC, USA, willow oak is living far below its thermal maximum, as its range extends across much of the southern US. Therefore, I predicted that warming would enhance growth rates in trees treated for herbivores at hotter sites. In contrast, I expected that cooler trees would grow less overall and would benefit from the insecticide treatment less because they had lower baseline herbivore abundances [22].

Herbivore abundance

To determine which arboreal herbivores were most abundant, and, therefore, had the potential to act as pests, I used a funnel beat sampler [28] in August 2013 and 2014. The

sampler is a 30.5-cm diameter funnel attached to a 2.5-m pole, with a hinged lid that beats arthropods from foliage into the funnel, where they fall into alcohol in a sampling jar fixed to the bottom of the funnel. I sampled foliage along the lowest part of crowns of study trees in 12 locations. Arthropods were stored in 95% ethanol and identified to the lowest taxonomic level possible without dissections or slide mounts (usually family or genus) under a dissecting microscope.

To determine how herbivores responded to temperature, I did more targeted counts of the two most abundant herbivore taxa from funnel beat samples, *Parthenolecanium* scale insects and spider mites (see *Results*). Both of these groups include chronic herbivores that live on leaves or stems their entire lives. To measure their abundances, I collected one 30.5 cm branch from each cardinal direction of the bottom, outer canopy of each study tree in August 2013 and June 2014. On these dates, I counted *Parthenolecanium* scale insects on 5 leaves per branch using a dissecting microscope. In March 2015, I collected, then counted overwintering *Parthenolecanium* scale insects on 12 30.5-cm branches from the bottom, outer canopy of each study tree. Because *P. quercifex* and its congener *Parthenolecanium corni* are both willow oak residents, are indistinguishable in nymphal stages, and feed on the same part of trees (phloem), counts of these species are pooled. In August 2013, I counted spider mites on the same leaves on which I counted *Parthenolecanium*.

Photosynthesis

I measured gas exchange and fluorescence with a LI-6400XT Portable Photosynthesis System equipped with a 6400-40 leaf-chamber fluorometer (LI-COR, Inc., Lincoln, Nebraska). I measured maximum light-saturated photosynthesis (hereafter “photosynthesis”), conductance, and transpiration on three full-sun leaves with no chewing damage on three severed branches in floral picks [29]—i.e., conical, water-filled containers— per study tree between 0800 and 1300 from July 7-19, 2014, on sunny, dry days. To prepare branches, I severed them from the tree, immediately re-cut them underwater, and, still underwater, inserted them in floral picks. During gas-exchange measurements, I held leaf chamber CO₂ concentration at 400 $\mu\text{mol mol}^{-1}$, vapor pressure deficit between 1 and 2 kPa, block temperatures at 30 °C, and used a saturating light intensity of 1200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. To determine if water deficits affected gas exchange, I used a pressure chamber (model: 1505D, PMS Instrument Company, Albany, OR, USA) to measure branch water potential on the same days I measured gas exchange. I haphazardly collected three bottom, outer branches from the north side of each tree, pressurized those branches, and recorded the pressure required to release water from the xylem.

I measured the maximum quantum efficiency of photosystem II (Fv/Fm), a common measurement of plant stress [30], on the same days I measured gas exchange. I cut 3 branches per tree, immediately re-cut branches under water, and then put them in floral picks. Leaves were dark-adapted in a cooler with ice packs (which cooled the air but did not contact leaves) for at least 4 hours. I measured Fv/Fm on 3 leaves per tree in a darkroom.

Growth

I measured DBH of each tree 1.4 meters above the ground in March 2014, October 2014, and June 2015. Because the growing season is between April and October in the study region, my goal was to capture total yearly growth in 2014 and delayed effects of the 2014 temperatures [31] on spring growth in 2015. I used DBH to calculate basal area (πr^2) and basal area growth (difference between starting and ending basal area) in 2014 and 2015, the years after the oil treatment was implemented. Branch growth reacts immediately to abiotic stressors [32] and is, therefore, a useful measurement of tree responses to environmental change. To determine stem growth in 2014 and 2015, I collected 12 branches from the bottom, outer branches of each study tree in March 2015. To determine spring growth in 2015, I measured branches from apical tips to terminal bud scars. To determine total 2014 growth, I measured stems from the terminal bud scar to the penultimate bud scar.

Carbon sequestration

Because both herbivores and warming affected tree growth, I wanted to determine the extent to which each of these stressors reduced carbon sequestration. To do this, I first used a species-specific allometric equation for willow oak [33] to estimate dry weight for study trees in March and October 2014. Then, I used methods in Nowak et al. [34] to estimate total carbon storage per tree from dry weights. The total carbon sequestered by study trees in 2014 was calculated as the difference between March and October carbon storage estimates.

To estimate how urban warming affected carbon sequestration by willow oak across the entire focal city, I used the same thermal map and tree inventory used to select study sites. From this map, I extracted surface temperatures at all city-owned willow oaks in Raleigh. I then determined the relationship between mean growing season air temperatures (April to October) and surface temperatures at study sites using simple linear regression. I used the resulting regression equation to project 2014 air temperatures at all willow oak trees in the tree inventory, i.e., all willow oaks managed by the local, municipal government. I used multiple regression to model the relationship between air temperatures at study sites, tree DBH at the beginning of the growing season, and estimated carbon sequestration. I used this regression equation (carbon sequestration = a *air temperature + b *DBH + intercept) to estimate total carbon sequestration by willow oaks in the tree inventory. I then set air temperatures to the lowest observed value in 2014 at study sites (23.4°C) to estimate carbon sequestration by willow oak with minimum urban warming. The difference between these two sequestration estimates (based on observed urban warming and minimum urban warming) represents the amount of carbon sequestration lost due to urban warming intensification.

While I could not directly estimate the effects of herbivory on biomass, I could determine the proportion of total tree biomass in tissues affected by herbivores, i.e., new branch biomass. I used a genus-specific allometric equation [35] to estimate total new branch biomass in each study tree. I used the same methods as above to convert branch biomass to carbon storage. Then, I calculated the proportion of total tree biomass stored in new branches

as an estimate of the carbon stored in tissues subject to growth reduction by *Parthenolecanium* scale insects.

Analyses

Analyses were performed in RStudio [36]. The ibuttons recorded unreasonably high midday temperatures (45°C+). I thought this to be associated with solar radiation heating ibuttons directly, such that measurements did not represent air temperatures [37]. To avoid biases introduced by these readings, I extracted temperatures between 1800 and 2000 hours and used these in all downstream analyses. These temperatures represent the urban heat island effect, which rises steadily after 1500 and levels off around 2000 hours [38], without capturing confounding effects of solar radiation. I calculated average temperatures in the *plyr* package [39] during spring and/or summer months I thought most biologically relevant to each response variable. Details about thermal predictors included in each analysis are located in table S1. In 2013, ibuttons failed at 2 sites, and, in 2014, an ibutton failed at one site. These sites were discarded from analyses. In 2014, an additional site was removed from the study due to a dissatisfied homeowner.

Parthenolecanium scale insects and spider mites (Acari: Tetranychidae) were the most common herbivorous arthropods. I analyzed their abundances relative to temperature and oil treatment with mixed effects models specified to fit the probability distributions associated with each response. In all analyses, I used linear models when possible and moved to more complex models (generalized linear models) when necessary. In all models,

temperature and oil treatment (yes/no) were specified as fixed effects and site was specified as a random effect. I modeled *Parthenolecanium* scale insect and spider mite abundance from 2013 using generalized linear mixed effects models (GLMER) with Poisson distributions and log link functions in the *lme4* package. In these models, I included sample identity as a random effect to correct overdispersion. I modeled *Parthenolecanium* scale insect abundance from 2014 and 2015 (log+1) using linear mixed effects models (LME) in the *nlme* package [40]. I did not record temperatures in 2015. Therefore, I used growing season temperatures in 2014 as a predictor of *Parthenolecanium* scale insect abundance in 2015. All R^2 values were derived from formulae for marginal and conditional R^2 .

I analyzed all gas exchange measurements and Fv/Fm with linear mixed effects models with temperature and oil treatment as fixed effects and tree nested within site (for gas exchange) or branch nested within tree nested within site (for Fv/Fm) as random effects. I $1/x$ transformed fluorescence (Fv/Fm) to improve residuals. To determine if water deficits drove gas exchange, I tested for a correlation between water potential and light-saturated photosynthesis using simple linear regression.

To determine how temperature and insect removal affected tree growth, I used linear mixed effects models with branch growth and basal area growth in each year as responses and temperature and oil treatment as fixed effects in the *nlme* package. For basal area growth, I specified site as a random effect, and for branch growth I specified tree nested within site as a random effect. Trees with bigger original DBH grow more in other systems [41, 42], so I included original basal area as a covariate in the model for basal area growth. Because basal

area growth and photosynthesis were lower due to warming, and branch growth tended to be lower though was not significantly affected by warming, I wanted to test if photosynthetic declines drove growth declines. To do this, I used simple linear regressions with mean photosynthesis per tree as the predictor and basal area and branch growth from 2015 as responses.

Results

Herbivore abundance

Parthenolecanium scale insects and spider mites were the most abundant herbivorous arthropods in funnel beat samples when counts are summed over both years (figure S1). Together, they made up over 65% of herbivores captured in funnel beat samples. In all years, hotter sites had significantly more *Parthenolecanium* scale insects (2013, figure 1a, Wald $Z=1.62$, $p=0.043$; 2014, figure 1b, $F_{1,16}=4.88$, $p=0.042$; 2015, figure 1c; $F_{1,16}=4.74$, $p=0.045$), such that the hottest site had 7 times more *Parthenolecanium* scale insects on average than the coolest site at which I detected *Parthenolecanium* scale insects. Trees treated with oil had half as many *Parthenolecanium* scale insects on average than untreated trees ($p<0.05$ in all years; for detailed statistics, see table S1). Similarly, hotter trees had up to 56 times more spider mites than the cooler sites; (figure 1d, Wald $Z=2.14$, $p=0.003$), and trees treated with oil had one third as many spider mites than untreated trees ($p<0.001$; table S1).

Photosynthesis

Light-saturated photosynthesis (figure 2a; $F_{1,16}=4.79$, $p=0.044$), conductance ($F_{1,16}=5.53$, $p=0.032$), and transpiration ($F_{1,16}=4.69$, $p=0.046$) declined linearly with warming but were not affected by oil treatments ($p>0.05$; detailed statistics in table S1). Although tree water deficits ranged from mild to moderate (PMS Instrument Company Guide) with stem water potential measurements ranging from -0.91 to -2.46 MPa, water potential did not significantly affect light-saturated photosynthesis ($F_{1,36}=0.03$, $p=0.865$). Neither warming ($F_{1,16}=1.09$, $p=0.311$) nor oil treatment ($F_{1,17}=1.04$, $p=0.322$) affected fluorescence (Fv/Fm).

Growth

As expected, basal area growth rates were higher for trees with larger basal areas at the beginning of each year (2014: $F_{1,16}=15.62$, $P=0.011$, 2015: $F_{1,16}=5.15$, $P=0.037$). Urban warming was associated with less basal area growth in both years (2014: figure 2b, $F_{1,16}=5.36$, $p=0.034$; $F_{1,16}=5.87$, $p=0.028$). Lower light saturated photosynthetic rates were associated with smaller basal area growth (figure 2c; $F_{1,34}=7.99$, $p=0.008$). Oil treatments did not affect basal area growth (2014: $F_{1,16}=0.34$, $p=0.566$, 2015: $F_{1,16}=0.01$, $p=0.908$). In contrast to basal area growth, 2015 branch growth was not affected by warming ($F_{1,16}=2.03$, $p=0.173$), oil treatments ($F_{1,17}=2.56$, $p=0.128$), or photosynthesis ($F_{1,34}=0.55$, $p=0.795$). In 2014, branch growth was not significantly affected by warming ($F_{1,16}=0.07$, $p=0.795$) but was increased by herbivore removal ($F_{1,17}=4.60$, $p=0.047$). In summary, warming decreased

basal area growth, and this effect was likely mediated by reduced photosynthesis. Arthropod pests did not affect basal area growth, but did reduce branch growth.

Carbon sequestration

Because urban warming reduced growth of study trees, I scaled these effects up to estimate how urban warming affected carbon storage by municipal willow oak in Raleigh. Urban warming reduced carbon sequestration of Raleigh, NC willow oaks by 34.7 metric tons in 2014 (figure 3). This represents a 15.6 % reduction in carbon sequestration due to the direct effects of warming on trees. The effect of herbivores on carbon storage was negligible, since they reduce growth only in new branch tissue, which accounts for a mere $0.45 \pm 0.05\%$ (mean \pm SEM) of total carbon stored by willow oak.

Discussion

Urban warming reduced tree growth, such that citywide carbon sequestration by willow oak was an estimated 15.6% lower due to the urban heat island effect. Therefore, these results show that warming in cities can significantly reduce the extent to which urban forests act as carbon sinks. Current estimates, which do not incorporate warming, likely overestimate carbon storage in cities, where trees are the primary repositories of carbon [43].

A key question is why urban warming reduced tree growth and carbon sequestration. One potential explanation relates to the influence of warming on herbivorous pests. As

predicted, urban warming dramatically increased arthropod pest abundance on study trees. However, experimental removal of pest arthropods through oil treatments successfully reduced their abundance, enabling us to separate the effects of temperature and herbivores on tree growth. These results revealed that herbivore effects were confined to new branches, and thus, accounted for less than 1% of tree carbon sequestration losses associated with urban warming. Together, these results point to a future in which even trees that are largely unaffected by higher herbivore abundance will store less carbon as climates warm.

Because this study site was located far north of willow oak's southern range limit, I expected that urban warming would increase its growth. Previous work on mature trees has shown that warming can benefit species at mid to high latitudes, where photosynthesis and other processes necessary for growth and reproduction are temperature limited [8]. Warming can also increase growing season length, leading to more growth per season [44]. While these mechanisms could have benefitted the trees in the present study system, net effects on tree growth were negative.

This study provides evidence that less growth at hotter sites was due to water deficits, consistent with drought studies [45]. Stomatal conductance declined linearly with urban warming. Stomatal conductance is regulated by water relations in many tree species, including willow oak [46]. When trees experience water deficits, they close their stomata to reduce water loss through transpiration. When I measured plant water deficit directly, I did not find a relationship to urban temperature. However, stomatal responses to water deficit can be delayed by hours or even days [47,48], such that water deficit at other time points,

during which I do not have data, could have reduced stomatal conductance at warmer sites. An alternative is that water deficit was not the mechanism for reduced growth at hot sites, and stomatal regulation of water status was sufficient to prevent a decline in water potential. Thus, more controlled experiments are required to test water deficit as a mechanism for tree growth declines in urban heat islands.

This study represents a highly conservative view of how further global and urban warming, and resulting herbivory, will affect urban forests. Oak (*Quercus*) contains some of the tree species most resilient to water deficits [49] and disturbance [50] worldwide, and urban tree species are selected for resilience. Of urban oak species, the study species willow oak is one of the most resilient, if not the most resilient, to herbivory [51]. Further, this study was located at the northern edge of willow oak's range, where warming should have its least negative effects [52]. More sensitive trees, such as red maple, and trees living at the hotter extremes of their geographic ranges, may have even stronger negative responses to stressors associated with global change.

Carbon storage losses due to urban warming should compound over years, leading to more dramatic effects on carbon storage. Bigger trees grew more in this study, which is a common pattern across tree species and habitats [41, 42]. When urban warming reduces tree growth, it also reduces tree size at the beginning of the next growing season. In turn, smaller trees should grow less in that year, leading to shallower growth trajectories for trees in hotter habitats. These compounded losses could substantially reduce the potential of urban forests to act as carbon sinks. In a recent estimate, trees harbored 97.3% of carbon stored in

aboveground urban biomass [43]. Urban forests in the present study region, the Southeastern U.S., store more carbon per hectare than urban forests in any other region, according to the most recent estimate [53]. Willow oak, and species that respond as it does to urban warming, should sequester even less carbon due to the urban heat island effect in more southern cities with stronger urban heat islands, such as Atlanta, Georgia.

In this study's most conservative interpretation, urban warming reduced ecosystem services that promote human health [56] and regulate climate [57]. We increasingly rely on urban forests to provide these services as deforestation of more natural forests continues [58] and as more people move to cities where they benefit directly from urban trees. These results point to a future in which urban habitats will store less carbon than they do now due to urban and global warming. These results also highlight the utility of cities as large-scale natural climate experiments, in which sessile organisms are confined to different thermal environments in close proximity. The range of urban warming they experience parallels the extent of global warming expected regionally, outside the city, over the next several decades. To the extent that urban forests can thereby predict the future of rural forests, this study presents a grim picture of future carbon storage and points to the need for models that incorporate the effects of abiotic and biotic stressors on tree carbon storage.

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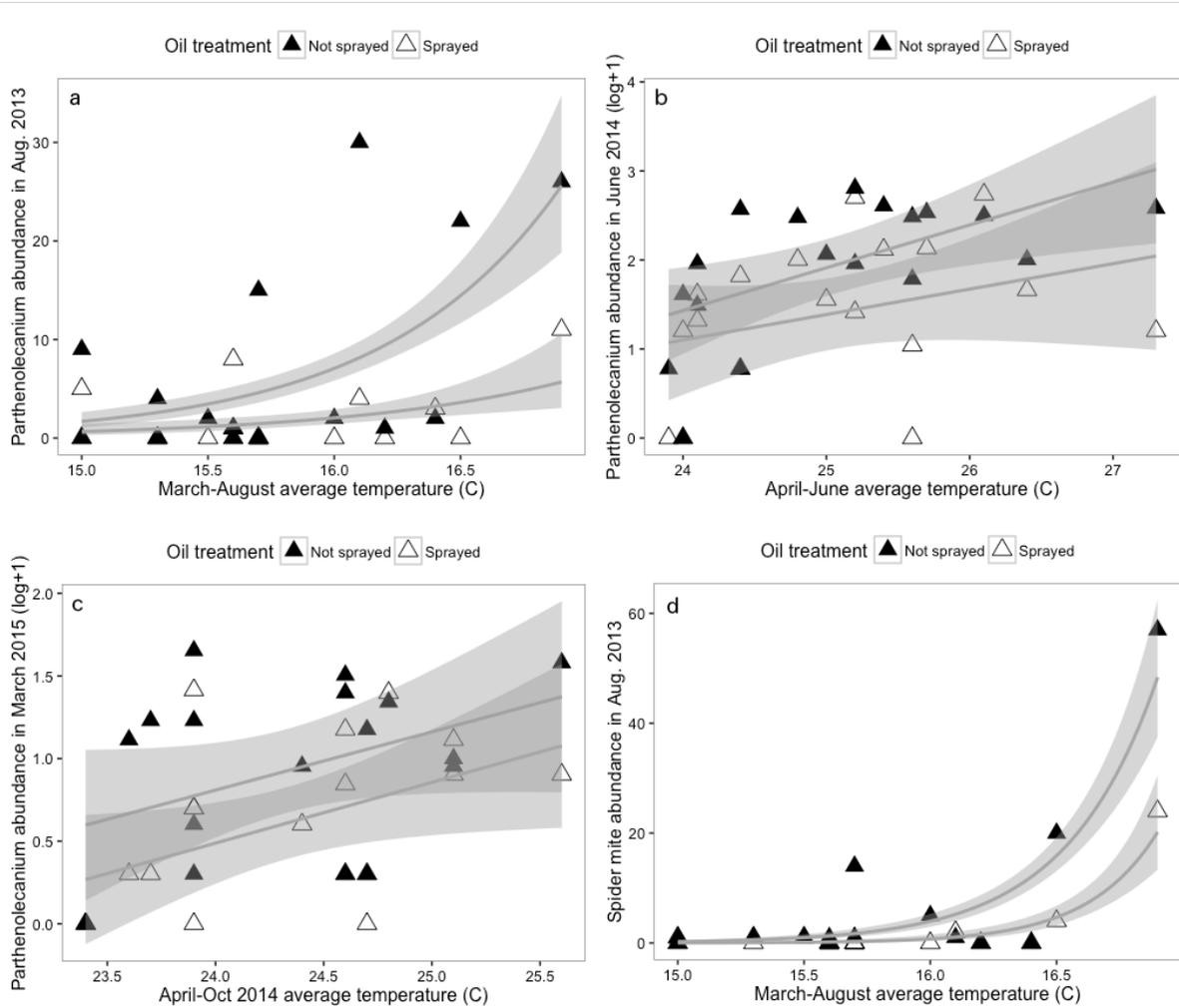


Figure 3.1 Urban warming increased chronic herbivore abundance, and oil treatments reduced chronic herbivore abundance. The two most common herbivorous arthropods on street trees in Raleigh, NC, USA, (a-c) oak lecanium scales (Coccidae: *Parthenolecanium* scale insects) and (d) spider mites (Tetranychidae) and were more abundant at hotter urban sites and less abundant due to oil treatments. Lines are means. Grey areas are 95% confidence intervals.

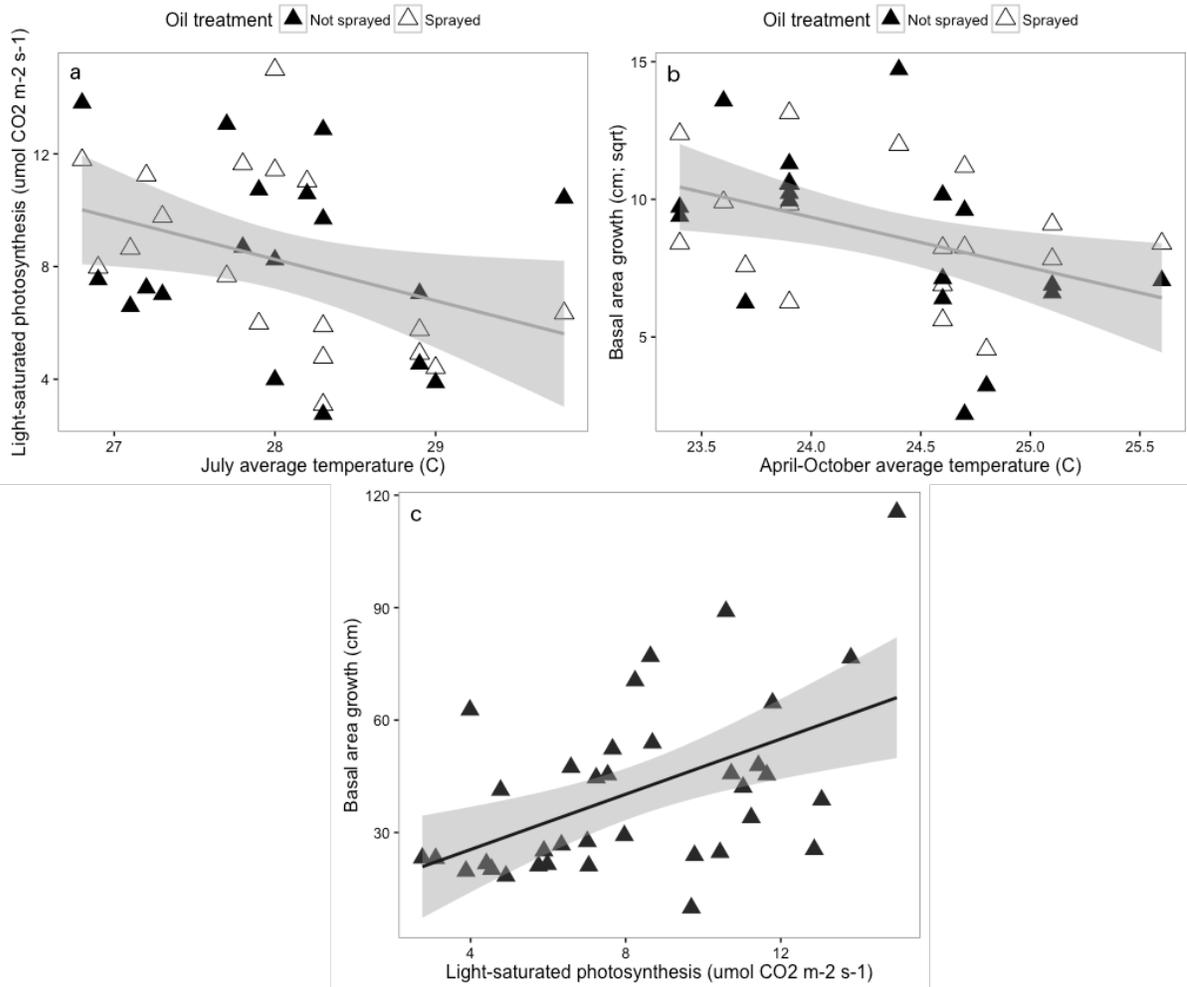


Figure 3.2. Urban warming reduced basal area (trunk) growth. Warming reduced (a) light-saturated photosynthesis and (b) basal area growth in 2014 (shown) and 2015. (c) Light-saturated photosynthesis and basal area growth were related, such that lower photosynthetic rates at hot site decreased growth. Lines are means. Grey areas are 95% confidence intervals.

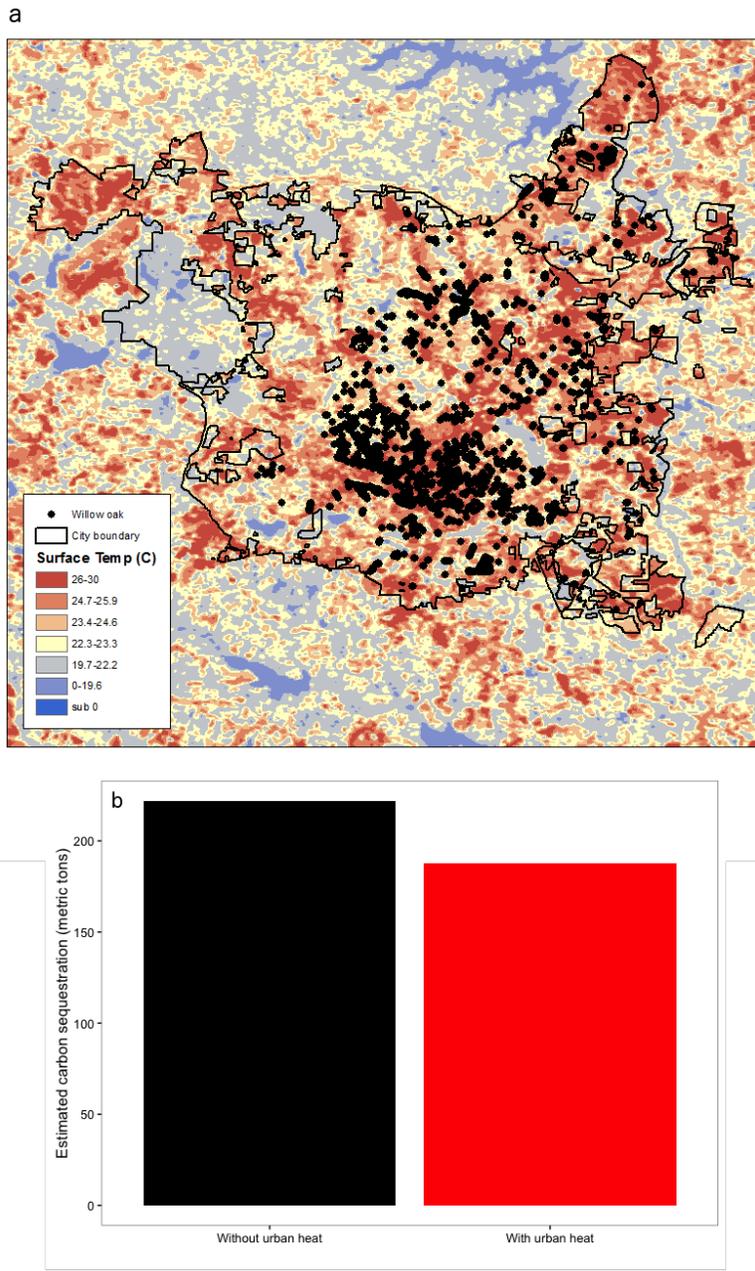


Figure 3.3 Carbon sequestration estimations across Raleigh, NC urban heat island. (a) Map of willow oak. (b) Estimated carbon sequestration by willow oak trees with and without the urban heat island effect.

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CHAPTER 4: EFFECTS OF INSECT PESTS AND WARMING ON TREE GROWTH HINGE ON WATER STRESS

Abstract

Trees are critical carbon sinks worldwide. It remains unclear, however, how trees at upper latitudes, where most aboveground carbon is stored, will respond to global change. This is in part because climate change imposes or/and exacerbates multiple threats to tree health, and experiments on mature trees to elucidate net effects of these threats are rare. I used a unique, urban warming experiment and a lab experiment to determine how three of the most potentially damaging global change factors for trees—warming, drought, and insect pests—affected growth and carbon storage of one of the most resilient urban tree species. In a previous study, I found that urban heat reduced tree growth. Here, I identified water stress as a potential mechanism for this by showing that trees at hotter urban sites are also more water stressed. In the laboratory, pests and warming only reduced leaf area and woody biomass in trees that were water stressed. In the absence of water stress, insect pests and warming had neutral or positive effects on trees. Together, I show that the future of urban tree function will depend on water availability and its interactions with insect pests and warming. Globally, this study provides evidence that even trees that should theoretically benefit from warming may acquire less biomass in response to secondary stressors, reducing their role as carbon sinks.

Introduction

Forests store 45% of terrestrial carbon [1]. Therefore, tree responses to global change will affect future climate stability. However, tree carbon stocks are the most uncertain variables in carbon models [1, 2], in part because multiple global change factors affect tree growth and function, such that net responses are difficult to predict. Further, field experiments on mature trees to elucidate these effects are often impossible to execute and are, therefore, rare.

Two of the aspects of global change with the biggest potential to influence tree growth are warming and precipitation. At mid to high latitudes, where most tree carbon is stored, warming alone has been predicted to increase tree photosynthetic and growth rates when trees have access to enough water [3, 4]. In contrast, where and when water is scarce, warming is predicted to reduce tree growth by increasing water stress [5]. Though these predictions are clear in theory and from small-scale experiments on seedlings and saplings, we lack the experimental evidence needed from mature trees to predict how future warming will affect tree growth and, in turn, carbon storage.

Warming and water stress can also increase arthropod pest abundance on trees, making the net effects of warming on tree carbon storage even more difficult to predict [6]. Species that kill trees, such as the mountain pine beetle, outbreak more often now than ever [7], and higher outbreak frequencies have been linked to climate change [8]. Similarly, chronic arthropod herbivores—non-outbreak species that reduce tree growth but typically do not kill trees—have become more abundant due to global change. This is in part due to warming and, in theory, could also be due to or exacerbated by water stress. Warming below

ectothermic species' thermal maxima increases metabolic rates and, in doing so, increases survival [9] and fecundity [10, 11]. Similarly, plant water stress can increase nitrogen availability to arthropod pests [12], increasing their abundances.

Urban warming gradients provide a unique opportunity to evaluate independent and combined effects of warming, insect pests, and water stress on mature trees. The urban heat island effect is warming in cities caused by hard surfaces that absorb radiation from the sun and emit this energy as heat. Within cities, trees have been planted and warmed by the urban heat island effect, often for decades. Urban warming increases temperatures from 1-12°C [13], levels expected across much of the world over the next century [14]. Further, urban warming increases chronic insect pest abundance [10, 15-17] and water stress [16], providing an opportunity to determine net effects of prolonged warming and the key stressors associated with it on the extent to which mature trees sequester carbon.

In a recent study, I found that urban warming in a temperate city was associated with less street tree growth and carbon sequestration (carbon storage per year). These declines were contrary to predictions. The focal species, willow oak *Quercus phellos*, was living near the northern edge of its range, where warming should have increased photosynthesis and growth [4]. Here, I explore the possibility that growth declines across temperature were due to greater insect pest abundance and/or water stress. First, I used a factorial field experiment across an urban warming gradient to determine if urban warming was associated with water stress and if water stress was associated with higher arthropod pest abundance. Half of study trees were treated for arthropod pests to determine whether these herbivores affected tree

growth. In the laboratory, I manipulated temperature, water availability, and insect pests in a factorial experiment on saplings to determine mechanisms for less tree growth at warmer urban sites. Together, this study elucidates the combined and separate effects of three of the most imminent and potentially damaging global change stressors on trees.

Methods

Study system

The focal tree species for this study was willow oak, *Quercus phellos*, one of the largest, most commonly planted tree species in cities across the eastern US. The focal pest insect, *Parthenolecanium quercifex*, or the oak lecanium scale (Hemiptera: Coccidae), is the most common pest on willow oak. *Parthenolecanium quercifex* is univoltine. In spring, *P. quercifex* females live on branches and feed on phloem. From March-May, they convert their biomass into up to 3000 eggs that are deposited underneath their abdomens [18]. Nymphs move to leaves where they feed during summer. They return to branches to overwinter before leaf fall in autumn. Scale insects can reduce tree growth directly by feeding on sap and by promoting sooty mould on tree leaves, which blocks leaf light interception and photosynthesis [19].

Field experiment

Study design

Raleigh, NC, USA is a humid, temperate city located at 35.77°N 78.64°W. To choose study sites, I used a Landsat infrared layer of surface temperature in summer 2011. The Raleigh Department of Parks, Recreation, and Cultural Resources provided a map of Raleigh street trees that I overlaid with the thermal image. I chose 10 sites above median temperature and 10 sites below median, each of which contained 2 willow oak trees less than 30 m apart. Trees were 20.9±1.2 cm (mean ±SEM) in diameter at breast height. I used a mist blower (model: 451, Solo, Newport News, VA, USA) to treat one tree per site with horticultural oil (SuffOil-X, BioWorks, Victor, NY, USA) to remove scale insects and other small, soft-bodied chronic herbivores. I treated trees in July 2013, September 2013, February 2014, March 2014, April 2014, and May 2014 until leaves were wet. I used a concentration of 2 L per 100 L of water in March 2013, September 2013, February 2014, March 2014, and 1 L per 100 L of water in July 2013, April 2014, and May 2014. This created a split plot design with temperature (hot/cold) as the whole plot factor and oil treatment (yes/no) as the split plot factor.

To measure air temperature, I installed an ibutton thermocron data logger (model: DS1921G, precision: 0.5 °C, accuracy: ±1.0 °C, Dallas Semiconductor, TX, USA) on the northern, bottommost branch of one tree per site. I placed ibuttons on shady branches to reduce sun exposure associated with malfunctions in other studies [18] . To further prevent

malfunctions due to solar radiation and precipitation, I housed ibuttons in wall mounts (Dallas Semiconductor of Dallas, TX) inside 2.54-cm deep plastic cups.

Responses

To determine *P. quercifex* abundance, I collected 12 30.5-cm branches from the bottom, outer canopy of each study tree in March 15, 2015 using a pole pruner. On this date, *P. quercifex* were 2nd instars and co-occurred with a congeneric species *Parthenolecanium corni*. I pooled counts of these species, because they are indistinguishable in nymphal stages and both feed on phloem.

I measured midday stem water potential, a measurement of peak water stress in trees. I collected 3 bottom, outer branches from the north side of each study tree using a pole pruner from 1100 until 1400 on August 16, 2013, from July 29-30, 2014, and from August 29-30, 2014. Branches were placed in a pressure bomb (model: 1505D, PMS Instrument Company, Albany, OR, USA). I recorded midday water potential as the amount of pressure required for the xylem to release water, as in other studies [16] To determine tree radial growth, I measured diameter at breast height of each tree 1.4 meters above the ground in October 2014 and June 2015. I used diameters to calculate basal area (πr^2) and subtracted starting basal area from ending basal area to calculate basal area growth in early 2015.

Statistical analyses

I performed all analyses in RStudio [20]. Ibuttons recorded unreasonably high midday temperatures (45°C+) that I believe were due to solar radiation heating ibuttons [21]. I avoided the biases introduced by these readings by extracting temperatures between 1800 and 2000 hours. I used these in all downstream analyses. These temperatures represent urban warming, which rises after 1500 and levels off around 2000 hours [22], without capturing biases introduced by solar radiation. I used the *plyr* package [23] to calculate average temperatures for July 15-August 15, 2013, July 1-29, 2014, and August 1-29, 2014, approximately one month before each water potential measurement. In 2013, ibuttons failed at 2 sites. These sites were discarded from analyses. In 2014, an additional site was removed from the study due to a dissatisfied homeowner.

To determine how temperature affected water potential, I created linear mixed effects models (lme) for each date on which water potential was measured. I specified water potential as the response (3 values per tree), temperature during the 30 days prior to water potential measurements as a fixed effect, and tree nested within site as a random effect in the *nlme* package [24]. To determine if water potential was associated with higher *Parthenolecanium* abundance and if oil treatments reduced *Parthenolecanium* abundance, I used linear mixed effects models with average water potential per tree in July and August and oil treatment as fixed effects and *Parthenolecanium* average abundance per branch as the response in each model. When I detected significant interactions, I tested for the effects of water potential on treated and untreated trees separately in simple linear models as post-hoc

tests of how water potential affected *Parthenolecanium* abundance. To determine how water stress and herbivore removal affected tree growth, I used linear mixed effects models with average water potential per tree in July and August as fixed effects, original tree size as a covariate [25, 26], oil treatment as a fixed effect, and tree basal area growth as the response.

Laboratory chamber experiment

Study design

To determine how temperature, drought, and insect pests affected trees, I conducted a common garden experiment with a 3 by 2 factorial design in the North Carolina State University phytotron facility (<https://www.ncsu.edu/phytotron/information.html>). I purchased 56 bare root willow oak saplings from Rennerwood, Inc (Tennessee Colony, TX, USA) and grew them in 20.3-cm pots in Fafard 2P potting mix (Agawam, MA, USA) starting on April 5, 2013. When trees arrived, they were 28.42 ± 0.84 cm tall (mean \pm SEM). I fertilized each tree on April 11, June 20, and July 20, 2013 by placing 4 grams of 14-14-14 (N-P-K) Osmacote Classic (Geldersmalsen, The Netherlands) pellets in the soil. Directly after arrival, I treated saplings with Safer Brand Insecticidal Soap (Lititz, PA, USA) 185 mL/L H₂O to remove any insects.

I placed the trees in 2 (hot/cold) 2.4×3.7×2.1-m chambers with T-12, 1500 ma, cool-white fluorescent and 100 W incandescent lamps. To determine sunrise/sunset times in chambers, I used projected sunrise/sunset data for 2013 from the US Naval Observatory (http://aa.usno.navy.mil/data/docs/RS_OneYear.php). For one hour at the onset of sunrise

and sunset, I only lit incandescent bulbs to simulate twilight. To choose cold chamber temperatures, I used 1981-2010 Raleigh normal average temperatures measured at the NCSU weather station (URL: <https://www.ncdc.noaa.gov/data-access/land-based-station-data/land-based-datasets/climate-normals/1981-2010-normals-data>). I raised temperatures 4°C from cold chamber temperatures in hot chambers to represent temperature differences commonly observed within urban heat islands [13]. I raised temperatures every month during spring and reduced them each month starting in August to induce dormancy and mimic natural conditions.

Chamber temperatures necessarily deviated from Raleigh normals in the following ways. In April and May, I raised cold chamber temperatures by 3°C above normal to induce leaf out so that *P. quercifex* could establish on saplings in May (see below). From August to November, I lowered chamber temperatures more quickly than is represented in normals to induce overwintering, because, in my past experience, laboratory saplings take longer than urban trees to overwinter. In November, after leaves began to fall, I set both chamber targets to equal temperatures as outdoor temperatures reached the low chamber temperature limits. Temperature and sunrise/sunset times in chambers are detailed in table S1.

To impose drought-like conditions, I gave half of the trees in each temperature treatment pulsed water inputs. The other half of trees was saturated. I watered trees in the “saturated” treatment every two days with 2.6 liters of water or until soil was saturated. On days when saturated trees were watered, I measured soil moisture using a soil moisture sensor (model: SM150, Delta-T Devices, Cambridge, England, UK) in three randomly

selected pots per treatment in 3 haphazardly selected locations per pot. When average soil moisture of “pulsed” trees in either temperature treatment was below 5%, I watered trees in all treatments with 2.6 liters of water or until soil was saturated. I used a pulsed rather than a consistently sparse watering regime, because pulsed water inputs are common in urban habitats and because pulsed water inputs can increase insect pest abundances [12]. To determine how treatments affected midday water potential, I clipped the highest, lateral branch from each tree at the stem base and pressurized it with the same methods used in the field study on July 22 and September 9, 2013. On July 22, one tree was watered before water potential measurements. I discarded this data point.

When *P. quercifex* matured on field trees in May 2013, I collected egg sacs from 3 willow oak trees on the NCSU campus not included in the urban field study. I attached three egg sacs to half of the saplings in each laboratory treatment using elastic ties.

Parthenolecanium quercifex hatched in the laboratory from late May to early June 2013 and settled on leaves. In June 2013, when I observed no more *P. quercifex* hatching in the city or in the warming chambers, I removed the egg sacs.

Responses

On June 27, 2013 and September 10, 2013, I counted *P. quercifex* on 8 haphazardly selected leaves. On each of these dates, I counted the number of leaves on each study tree that were infested with *P. quercifex*. On June 27, I labeled five of the leaves I counted. I recounted *P. quercifex* on those leaves on July 27 and September 9, 2013 to determine how watering

regimes and temperature affected *P. quercifex* survival. To estimate *P. quercifex* abundance per tree for analyses, I calculated average *P. quercifex* density per leaf in June and September and multiplied these values by the number of infested leaves on each tree.

To estimate the total leaf area on each study tree, I first counted all leaves on each tree on April 11, June 27, July 27, and September 10, 2013. To estimate average area of individual leaves, I collected 5 leaves per tree and measured their area on a LI-COR Area Meter (model: LI-3100, Lincoln, NE, USA) on August 28, 2013. To estimate total leaf area per tree on each date, I multiplied average area per leaf by the number of leaves. I estimated leaf area acquired by each tree over the course of the experiment by subtracting estimated total leaf area in September from estimates in April. To determine the mechanisms for leaf area differences between treatments, I also calculated the number of leaves acquired over the course of the experiment (total leaves in September minus leaves in April) and the average leaf size per tree.

To determine plant biomass, I harvested all trees on December 11-12, 2013. I clipped each tree with pruners at soil to separate aboveground (shoot) from belowground (root) biomass. I washed soil from roots using water. I dried shoot and root biomass separately in paper bags in a drying room until late January 2014. I weighed dried shoot and root biomass of each tree. To calculate total biomass per tree for analyses, I summed shoot and root biomass.

I expected that pulsed water inputs would increase nitrogen concentrations and that this would increase *P. quercifex* survival and abundance. Therefore, to determine nutrient

concentrations, I returned the trees to the drying room and ground dry biomass in February 2014 using a grinder (model: 4 Wiley Mill, Thomas, Swedesboro, NJ, USA). Dried, ground biomass samples were deposited in the NCSU Environmental and Agricultural Testing Service, where carbon and nitrogen concentrations were determined using a Perkin Elmer 2400 CHNS Analyzer (Akron, Ohio, USA). To determine carbon to nitrogen ratios, I divided percent carbon by percent nitrogen. To estimate carbon sequestration, I multiplied percent carbon in shoot biomass with shoot biomass and percent carbon in root biomass with root biomass for each tree. I added these values to determine total carbon sequestered per tree. Data were missing from two shoot samples and one root sample.

Statistical analyses

I analyzed *P. quercifex* survival from June to July and from July to September using logistic regressions (glm) in the *stats* package [20] with average survival per leaf was the response and temperature and water treatment as predictors. I included total leaves on which *P. quercifex* was counted as weights in the models. I used ANOVAs to determine how treatments affected estimated *P. quercifex* abundance, which was log transformed to improve residuals.

To determine how treatments affected plant response variables of interest, I used ANOVAs for each response with temperature (cold/hot), *P. quercifex* (absence/presence), and water treatment (saturated/pulsed) as factors. Initially, I included a three-way interaction between all factors and progressively removed non-significant interactions. I modified this

analysis for models that did not meet ANOVA assumptions, as specified in subsequent paragraphs. Models for shoot biomass, root biomass, and total biomass, were square-root transformed. Models for percent nitrogen, percent phosphorous, and percent carbon were $\text{asin}(\sqrt{x/100})$ transformed. All transformations resulted in models that met ANOVA assumptions.

Models of water potential from both dates that included all predictor variables did not meet ANOVA assumptions due to unequal variances between treatments. Therefore, I first used non-parametric Kruskal-Wallis tests to determine if water treatments (saturated/pulsed) affected water potential. Then, I used 2-way ANOVAs to test for the effects of temperature and *P. quercifex* on pulsed and saturated trees, separately. I included interactions between temperature and *P. quercifex* and kept them in final models if they were significant. To improve residuals, water potential of saturated trees on both dates was $\log+10$ transformed.

In the full model for leaf area acquired by trees, 2 interactions were significant. I did not believe I had enough power to detect differences between all significant treatments using Tukey Honest Significant Difference (HSD) tests ($n=7$ for each treatment). Therefore, I used separate 2-way ANOVAs to test for the effects of temperature and *P. quercifex* on pulsed trees and saturated trees. I used these same methods to analyze total leaves (sqrt) acquired by trees. For analyses of total leaf area acquired, within the pulse-stressed treatment, I found a significant interaction between temperature and water treatment. Therefore, I used a Tukey HSD to test for differences between all treatments. I used a linear contrast to test the *a priori* hypothesis that *P. quercifex* only reduced leaf area on hot trees with pulsed water inputs. In

all analyses, leaf area acquired was log transformed to improve residuals. One tree was excluded from analyses because it was nearly dead, and I could not measure leaf area because leaves were too dry.

Because *P. quercifex* densities varied within treatments, I tested for the effects of *P. quercifex* as a continuous predictor on leaf area acquired, biomass, and carbon sequestration. I built separate, simple linear models for each treatment, response variable, and predictor. Predictors included average estimated *P. quercifex* per leaf, estimated *P. quercifex* abundance, and percent of leaves with *P. quercifex* in June, early in the experiment when *P. quercifex* was presumably feeding most.

Results

Field experiment

Treating trees for herbivorous arthropods did not affect water potential ($p > 0.05$ for all dates) or basal area growth ($p > 0.05$). Trees at sites with warmer air temperatures had lower water potentials (higher water stress) on both dates in 2014 (July: temp.: figure 1a, $F_{1,17}=4.46$, $p=0.049$, $R^2_c=0.09$, $R^2_m=0.43$; August: temp.: figure 1b, $F_{1,17}=10.47$, $p=0.005$, $R^2_c=0.16$, $R^2_m=0.58$) but not in 2013 (all predictors: $p > 0.05$). There was a significant interaction between water potential in July and oil treatment on *P. quercifex* abundance (figure 1c, $F_{1,15}=5.19$, $p=0.038$). Untreated, but not treated ($p > 0.05$) trees with higher water potential in July had more *Parthenolecanium* scale insects (July: $F_{1,17}=9.39$, $p=0.007$, Adjusted $R^2=0.32$), such that the most water stressed tree had 43 times more than the least stressed tree at which I

detected individuals. In the model with August water potential, oil treatments reduced *Parthenolecanium* scale insect abundance ($F_{1,16}=14.87, p=0.001$), but I detected no effect of water potential ($p>0.05$). Trees with higher water potential in August grew less in early 2015 (figure 1d, July: $p>0.05$; August: $F_{1,32}=10.71, p=0.003$, Adjusted $R^2=0.36$, with questionable data point, $p=0.041$).

Laboratory chamber experiment

More *Parthenolecanium quercifex* survived in hot than cold chambers from June to July (figure S1, temp.: $X^2_1=0.9, p<0.038$, water: $p>0.05$) but not from July to September (all predictors: $p>0.05$), indicating that, at least in this life stage, temperature, not tree water stress, increases *P. quercifex* survival, corroborating previous experiments [17]. Nutrients did not explain differences in *P. quercifex* survival across treatments. Trees with pulsed water inputs had significantly higher nitrogen concentrations and significantly lower C:N ratios in shoots (percent nitrogen: figure S2a, $F_{1,49}=124.65, p<0.0001$; C:N: figure S2b, $F_{1,49}=102.29, p<0.0001$) and in roots (percent nitrogen: figure S2c, $F_{1,50}=59.18, p<0.0001$; C:N: figure S2d, $F_{1,50}=36.52, p<0.0001$). There was a non-significant trend toward higher percent nitrogen in roots in saturated plants with *P. quercifex* ($F_{1,50}=3.88, p=0.054$). Neither water availability nor temperature affected total *P. quercifex* abundance (all predictors: $p>0.05$).

In July and September, trees with pulsed water inputs had significantly lower water potential (higher stress) than those in the saturated treatment (July: figure 2a, Kruskal-Wallis

$X^2_1=34.13, p<0.0001$; September: figure 2b, Kruskal-Wallis $X^2_1=39.19, p<0.0001$), which indicates that water treatments were implemented as intended. In July, within pulsed trees, the effects of temperature interacted with the effects of *P. quercifex* (temp. \times *P. quercifex*: $F_{1,23}=7.62, p=0.011$), such that cold trees with *P. quercifex* were more water stressed than without *P. quercifex*. Neither *P. quercifex* nor temperature affected water potential of saturated trees ($p>0.05$). In September, water potential was uniform across trees within water treatments, such that neither *P. quercifex* nor temperature affected water potential of pulsed or saturated trees.

Within saturated trees, temperature did not affect leaf area acquired ($p>0.05$). However, *P. quercifex* had a nearly significant positive effect on leaf area ($F_{1,25}=4.20, p=0.051$). Within trees that received pulsed water inputs, there was a significant interaction between *P. quercifex* and temperature (figure 3a, $F_{1,23}=6.84, p=0.015$), such that hot trees with pulsed water inputs and *P. quercifex* acquired the least leaf area (Tukey HSD $p=0.081$; Linear contrast $p=0.010$). Less leaf area in hot, pulsed trees with *P. quercifex* was due to lower leaf size and number. Within the pulsed water treatment, hot trees acquired fewer leaves ($F_{3,24}=46.53, p=0.012$). Temperature did not affect leaves acquired in the saturated treatment ($p>0.05$). However, saturated trees with *P. quercifex* acquired more leaves than those without *P. quercifex* (figure S3a, $F_{1,25}=5.90, p=0.023$), which indicates that, when saturated, trees can compensate for *P. quercifex*. In the model for leaf size, there was a significant interaction between temperature and *P. quercifex* (figure S3b, $F_{1,50}=6.89, p=0.032$), such that average leaf size was lowest on hot trees with *P. quercifex*. However, I did not detect significant differences between individual treatments using Tukey HSD.

Treatment effects on woody biomass mirrored effects on leaf area, such that the effects of warming and *P. quercifex* on tree biomass depended on water availability. Hot trees with pulsed water inputs had less shoot biomass than any other treatment (figure 3b, temp.*water: $F_{1,50}=11.65$, $p=0.001$). Hot, saturated trees had more root biomass than any other treatment (figure 3c, temp.*water: $F_{1,50}=12.79$, $p<0.001$). In the model for total biomass, there was a nearly significant three-way interaction between *P. quercifex*, temperature, and water treatment (figure 3d, $F_{1,47}=3.91$, $p=0.054$), such that, similar to leaf area acquired, mean biomass was lowest on hot trees with *P. quercifex* that were given pulsed water inputs. Carbon sequestration followed the same trend as total biomass, but only the interaction between temperature and water was significant (figure 3e, $F_{1,47}=21.52$, $p<0.0001$).

In analyses with *P. quercifex* as a continuous predictor, trees with a higher percentage of infested leaves had significantly lower total biomass, but only if they were in hot chambers and received pulsed water inputs (figure 3i; $F_{1,11}=-5.08$, $p=0.046$, Adjusted $R^2=0.32$). All other response variables followed this trend, notably shoot biomass (Figs. 3f-j; $F_{1,11}=4.26$, $p=0.063$, Adjusted $R^2=0.28$). *Parthenolecanium quercifex* per leaf and estimated *P. quercifex* abundance were not significantly associated with any of the response variables.

Discussion

Water stress is a major interactive factor reducing tree growth in urban heat islands. In the city, trees at hotter sites were more water stressed, and water stress was associated with

higher insect pest abundance and less tree growth. In the laboratory, water stress, insect pests, and warming combined to reduce tree leaf area and biomass. In the absence of water stress, neither warming nor insect pests negatively affected trees, which indicates that water stress turns these two features of global change into tree stressors.

For the first time, this study shows that certain insect herbivores only reduce tree biomass when trees are water stressed. Therefore, certain insect pests—even those that do not become more abundant with climate warming—will become more damaging to trees as trees become more water stressed with climate and land use change. This novel result is consistent with tree function studies that identify photosynthesis is a key mechanism by which trees compensate for herbivory. Phloem feeders, such as *P. quercifex* scale insects, remove photosynthate and, in doing so, stimulate photosynthesis [27]. However, compensatory photosynthesis requires that plants can open their stomata. These results are consistent with a model in which laboratory saplings that were water stressed could not increase photosynthetic rates in response to *P. quercifex* due to stomatal closure. In turn, *P. quercifex* reduced biomass accumulation in water stressed trees. As the physiological functions involved in this response—i.e. photosynthesis, stomatal conductance, and transpiration—are generalized across plants, these results suggest that many plant species will grow less in response to novel insect pests as plants become water stressed with climate change and urbanization.

Willow oak in Raleigh would accumulate more biomass due to urban warming if they were not water stressed. As predicted, warming only reduced biomass of water stressed

chamber saplings. This result is consistent with theory, other experiments on saplings [5, 28, 29], and observational studies on mature trees [30] that show trees living below their thermal optima grow more when warmed, but only in the absence of water stress.

If these results generalize, tree carbon storage will decline globally. Drought intensities and frequencies are expected to increase globally with climate change [31], and urban warming can induce tree water stress in oaks, like other tree species [16]. Further, trees worldwide are now living close to their hydraulic limits, such that further soil drying will lead to water stress, even in mesic habitats [32]. This study predicts that as water stress in trees increases worldwide, the extent to which insect herbivores reduce the carbon sink capacities of forests will increase.

As I found in another study [17], warming during spring and early summer increased *P. quercifex* survival in laboratory chambers. However, this was not driven by nutrients. Plants that have higher nitrogen concentrations are generally more nutritious for insects, which are nitrogen limited [33]. In the laboratory study, low water availability, not warming, increased nitrogen concentrations. Though water stress did not increase *P. quercifex* survival or abundance in the laboratory, this study leaves open the possibility that water stress is a mechanism for higher pest abundance in urban heat islands. *Parthenolecanium quercifex* had limited opportunities to benefit from higher nitrogen concentrations in water stressed trees in our study. First, many insects produce more eggs in response to higher nitrogen concentrations [11]. In the present study, *P. quercifex* remained nymphs, and, thus, did not produce young. Further, *P. quercifex* feed most as second instars (*Meineke, personal*

observation), and this life stage was not included in our study. Other *P. quercifex* life stages and other arthropod species may benefit from higher nitrogen concentrations in water stressed plants in urban heat islands.

This study is a highly conservative representation of the extent to which global change factors interact to reduce tree growth and carbon storage. Willow oaks were located in the northern part of their range, where warming should have increased growth in the absence of water stress. Further, willow oak is one of the most resilient tree species to drought [34] and insect pests [35]. Warming, water stress, and insect pests should have even stronger effects on less resilient species and or/and those living at their southern range margins. In the future, drought tolerance will likely serve as an adaptive trait, whereby trees can escape water shortages and intensified pest damage. Land managers should include drought tolerance as a factor in species selection, even in mesic forests such as those located in our study area, when a goal of management is to promote carbon storage.

Temperate and boreal forests are becoming more hospitable for many arthropod pest species. Trees in these forests are already subject to more frequent and catastrophic insect outbreaks. Our results predict that resident, native insects will become tree pests as urban heat islands and global climate change intensify. Further, trees will store less carbon as the climate warms, soils dry, and insect pests become more damaging, regardless of their abundances. These changing biotic effects should be measured for more insect and tree species and, subsequently, incorporated in carbon storage models.

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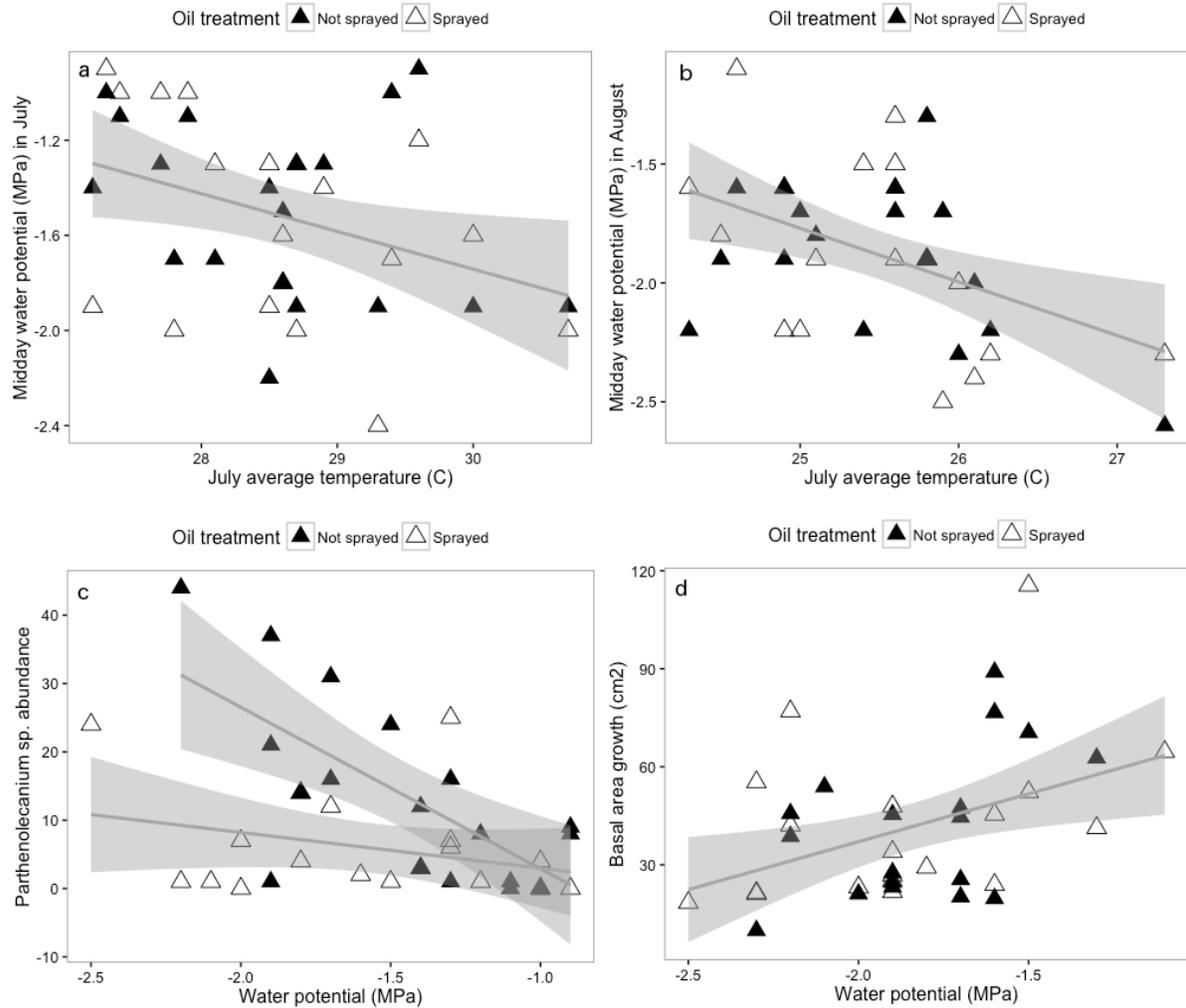


Figure 1. Correlations between warming, water stress, insect pests, and tree growth in the city. (a-b) Urban warming increased tree water potential (stress). (c) Trees with lower water potentials (higher water stress) had higher *Parthenolecanium* scale insect abundance and (d) grew less.

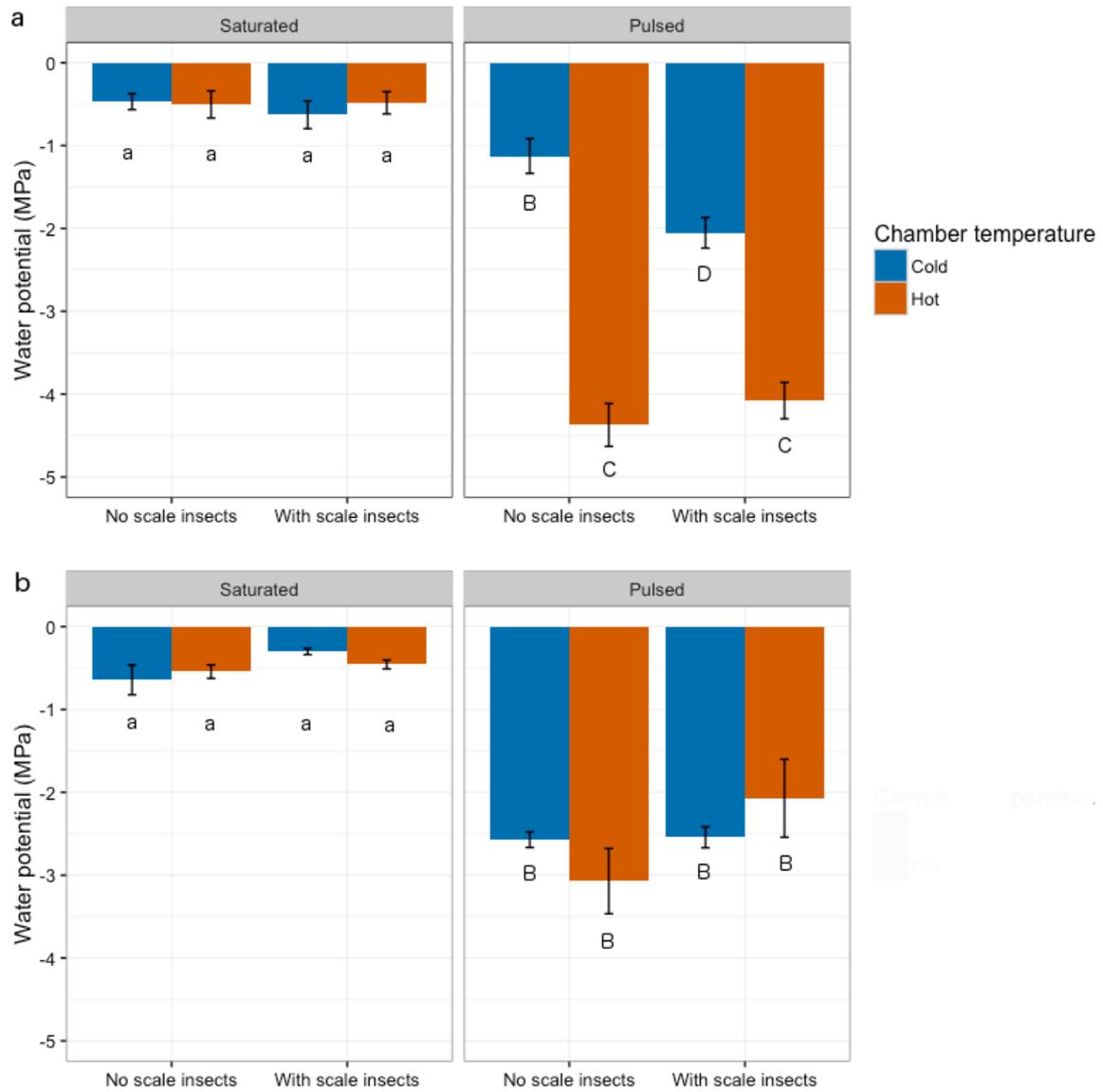
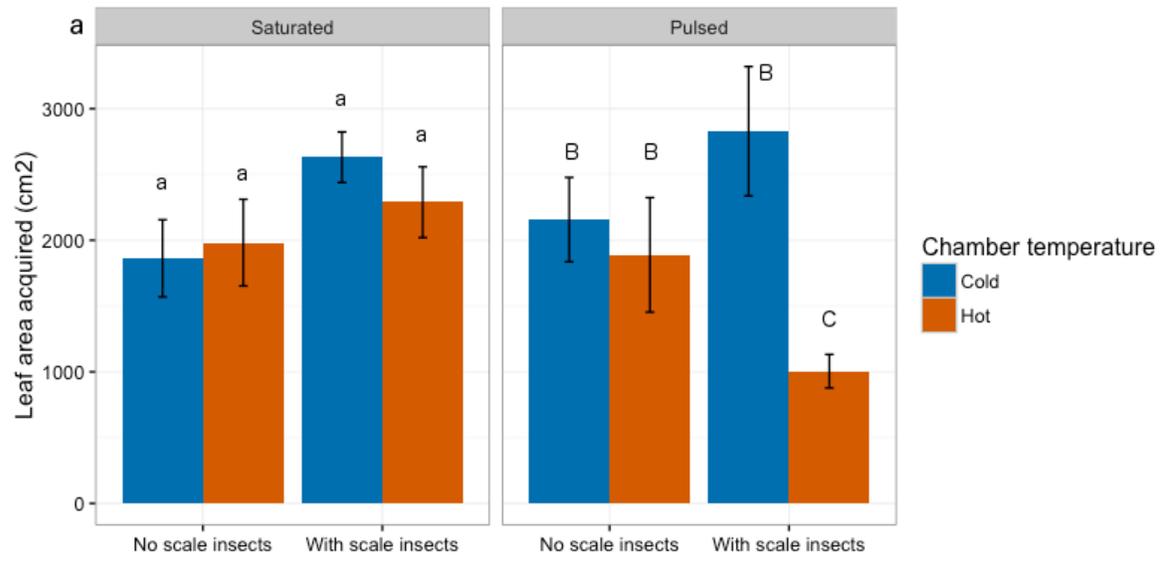
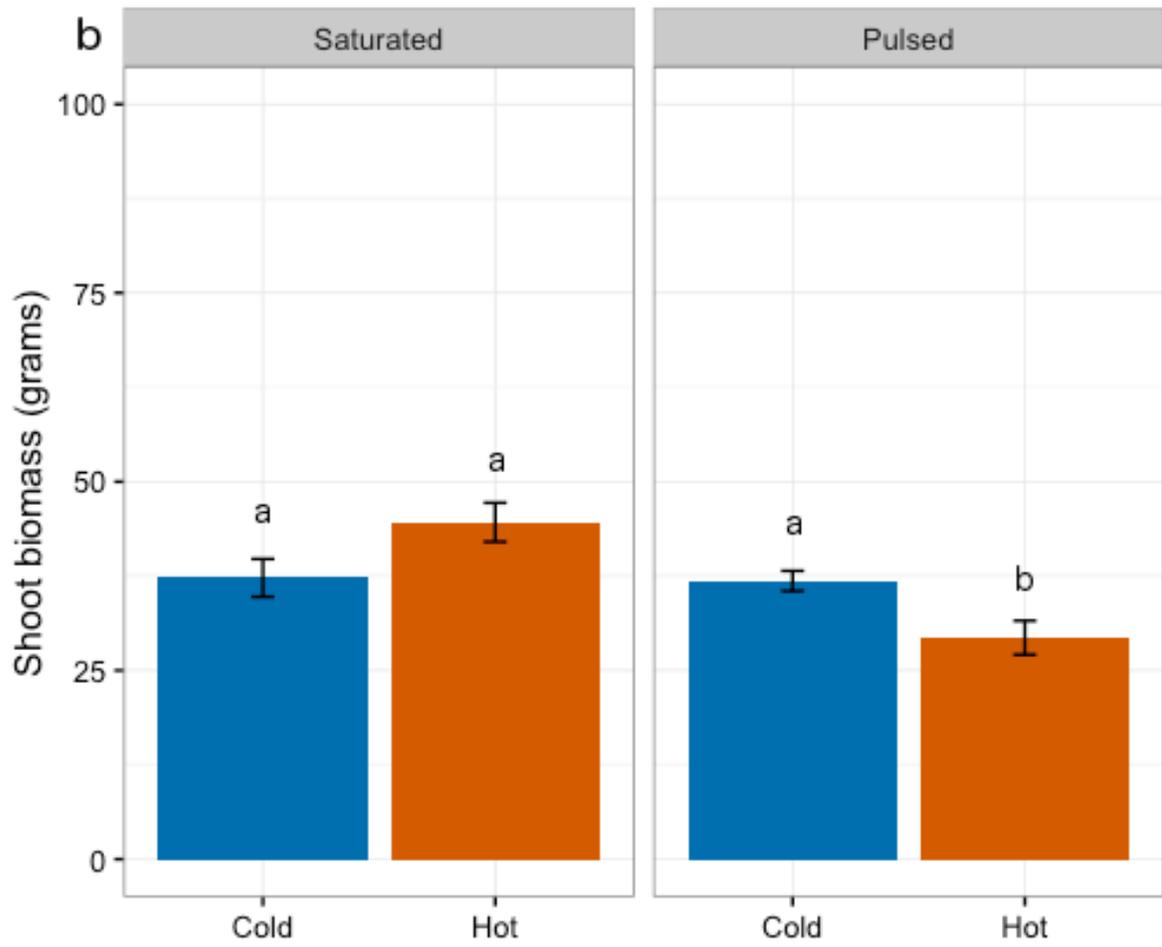
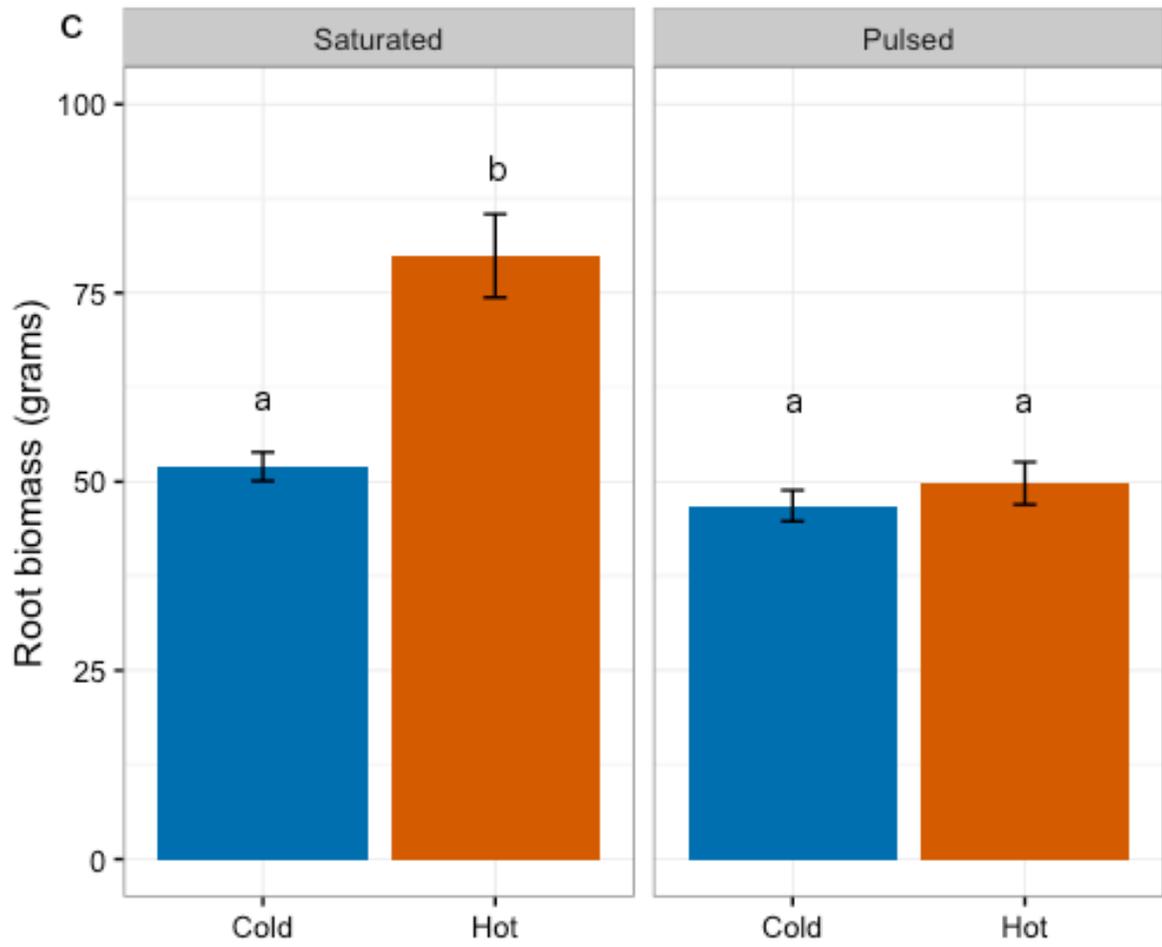


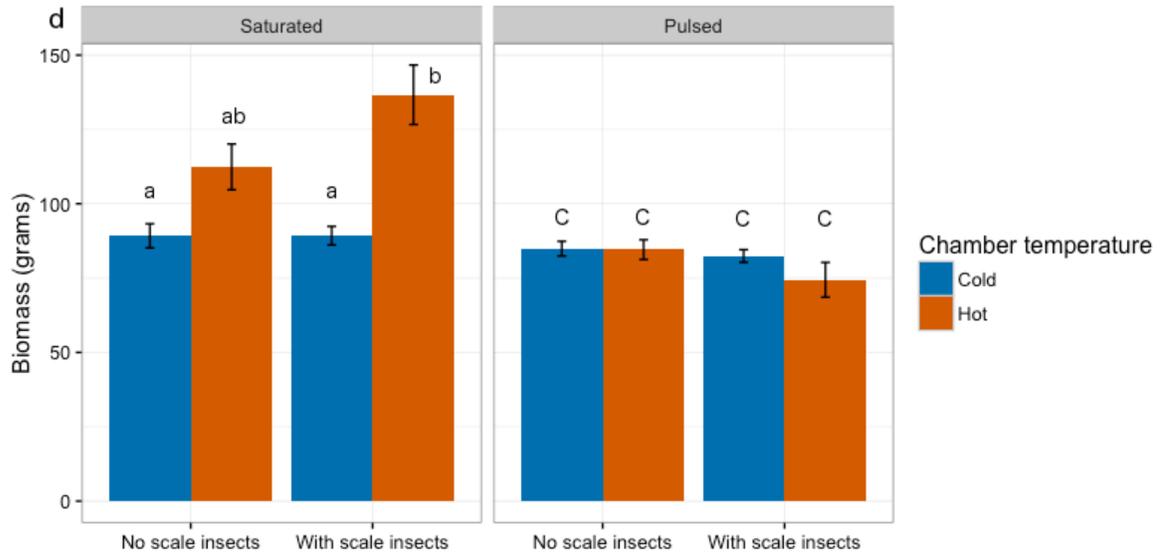
Figure 2. Effects of warming and insect pests on tree water stress. (a) In July, *P. quercifex* increased water stress, but only on trees that were relatively cool and, therefore, not as water stressed. (b) In September, pulsed trees were uniformly stressed. Letters refer to Tukey HSD tests.

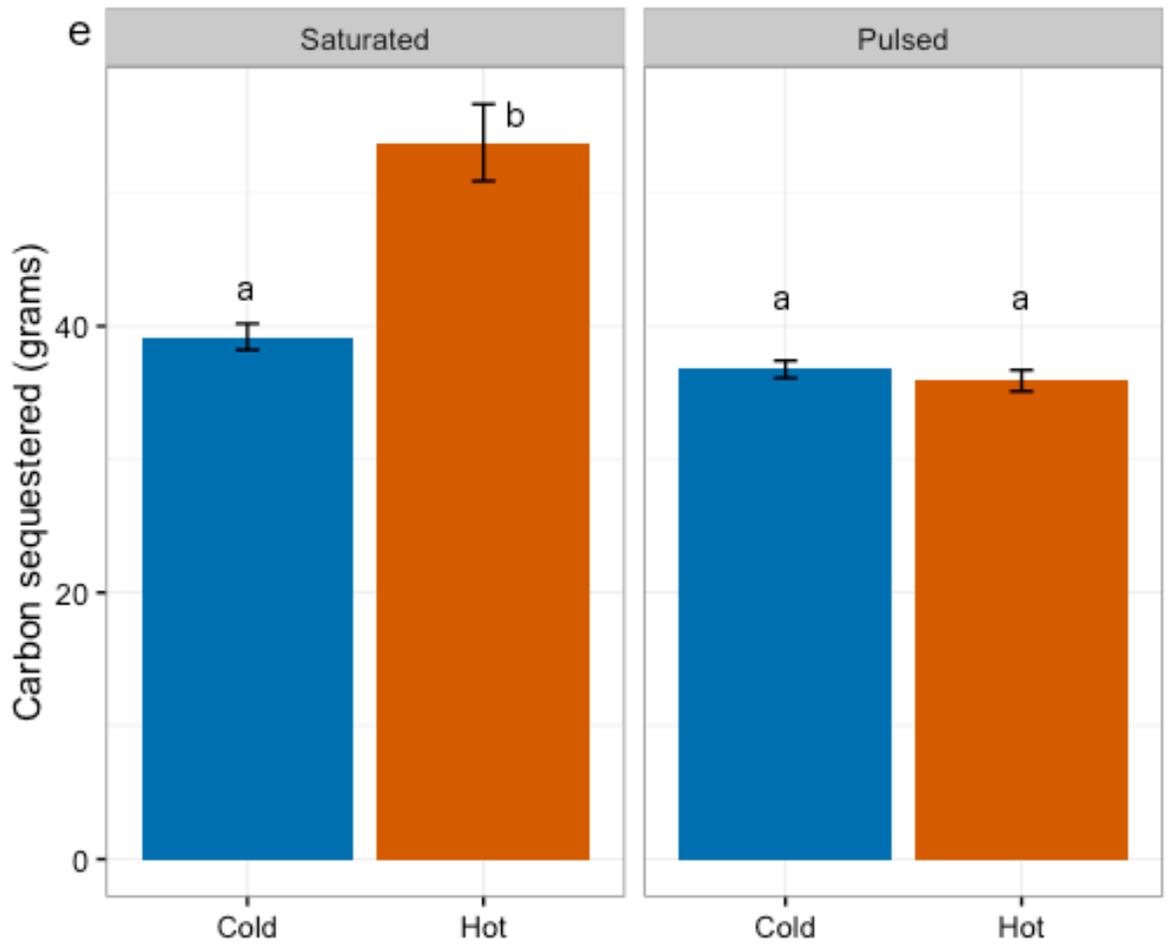
Figure 3. Effects of warming and insect pests on trees depended on water availability. (a) *Parthenolecanium quercifex* only reduced leaf area when trees were in hot conditions with low water availability. (b) Hot trees with pulsed water inputs had less shoot biomass than other treatments. (c-d) Hot, saturated trees had the most root and total biomass. (e) Hot, saturated trees sequestered the most carbon. (i) *Parthenolecanium quercifex* only reduced total biomass when trees were in hot conditions with low water availability. (f-h, j) All other responses also followed this trend. Only significant categorical predictors are shown, with the exception of (c), in which all treatments are shown due to a nearly significant three-way interaction. Letters refer exclusively to Tukey HSD tests except in (a), in which bars that were significantly different in linear contrasts are were also given different letters

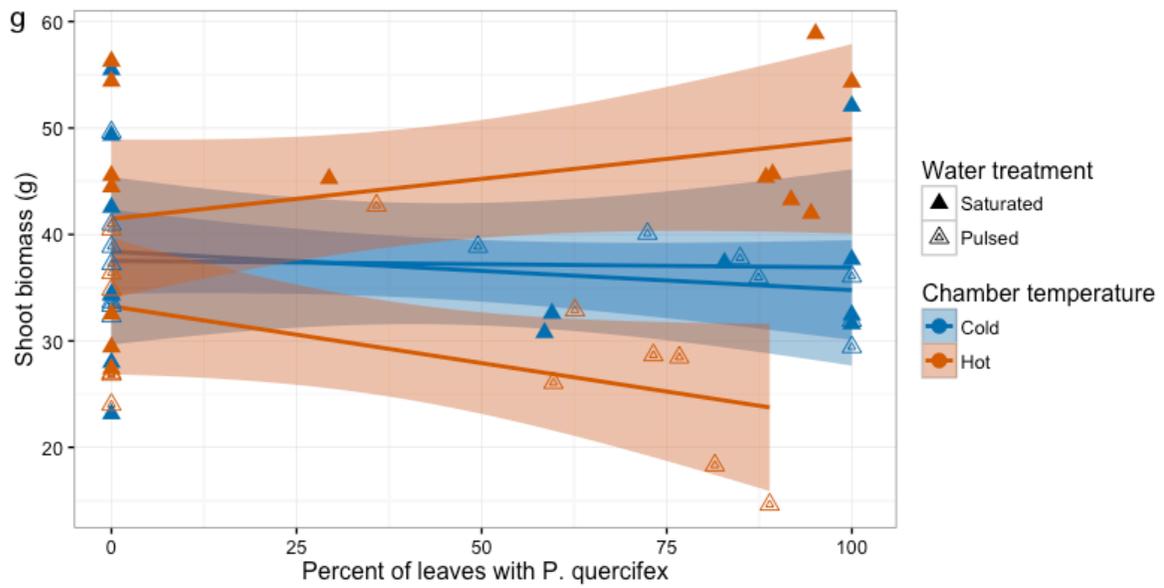
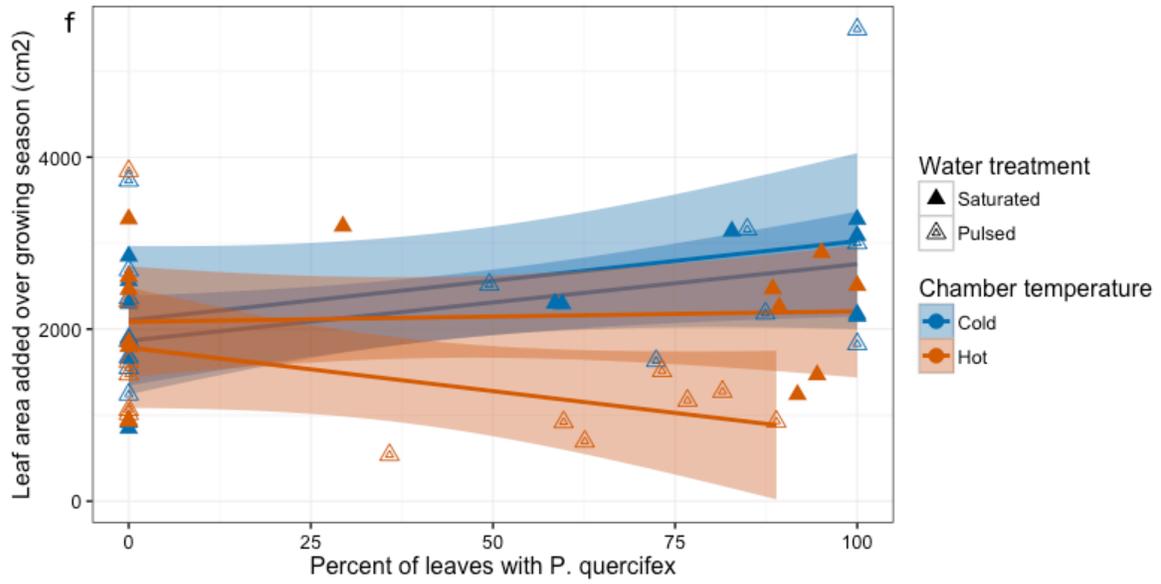


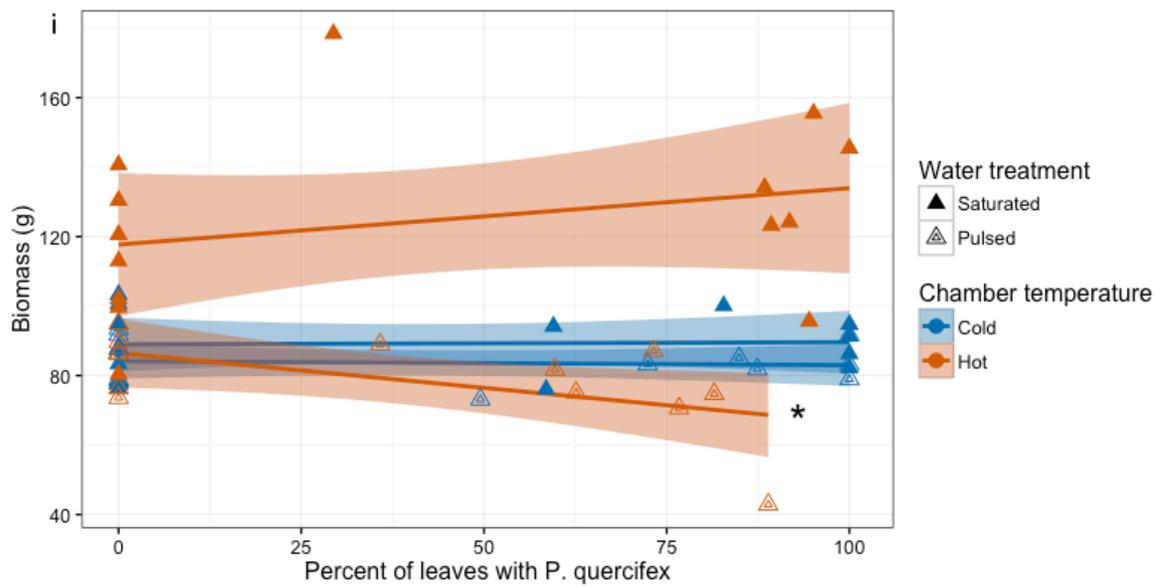
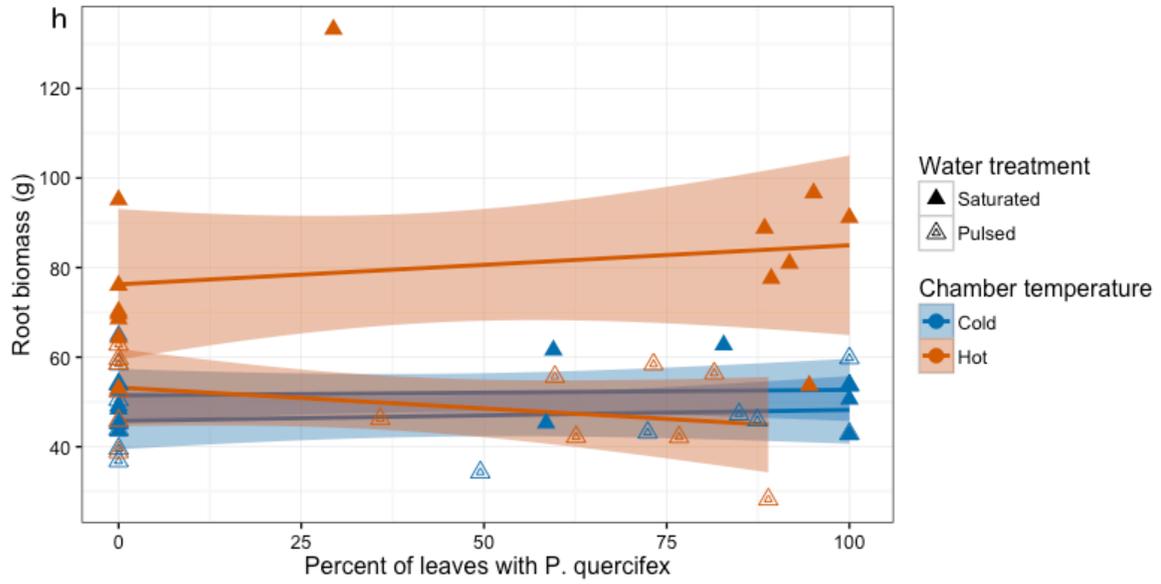


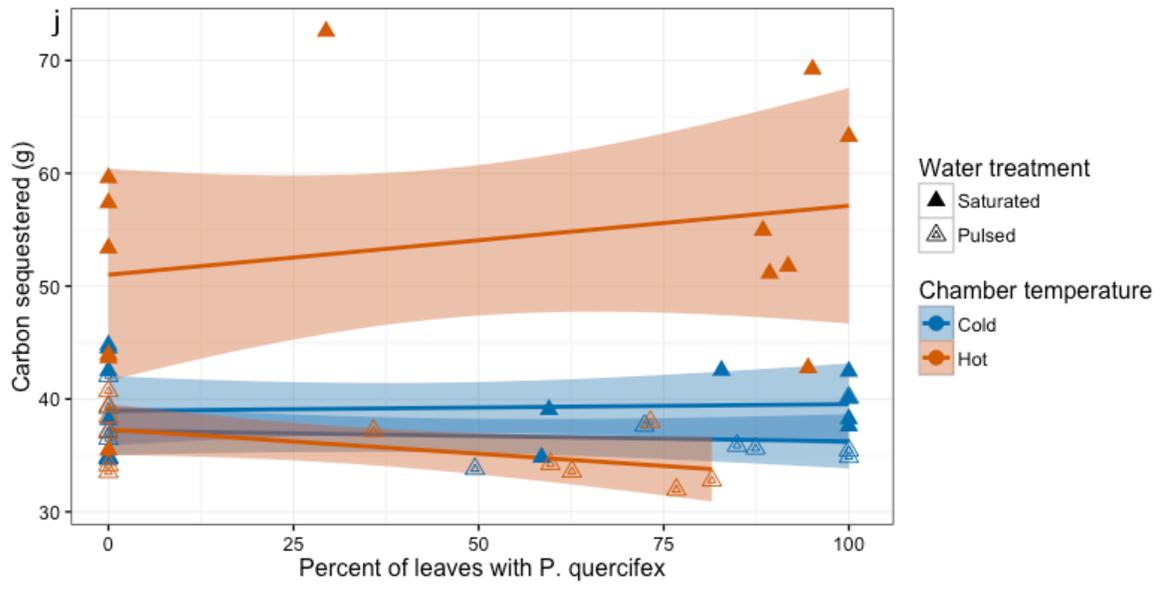












APPENDICES

APPENDIX A. SUPPLEMENTAL MATERIAL FOR CHAPTER 2

Parasitoid communities

Species of 5 parasitoid genera emerged from *P. quercifex*: *Blastothrix* sp., *Metaphycus* sp. (both Hymenoptera: Encyrtidae), *Coccophagus* sp. (Hymenoptera: Aphelinidae), *Eunotus lividus*, and *Pachyneuron* sp. (Hymenoptera: Pteromalidae). Individuals of *Pachyneuron* sp. on sticky cards were excluded from analyses since *Pachyneuron* sp. can be both a primary parasitoid (ovipositing inside scale insects) and a hyperparasitoid.

To test for differences between parasitoid communities at hot and cold sites, we compared communities that emerged from individual *P. quercifex* collected on April 16, 2012 (collection methods in main manuscript). I used nonmetric multidimensional scaling in PC-ORD 6 (McCune and Mefford 2011) to create a distance matrix of the number of parasitoids of each genus that emerged per *P. quercifex* at each site. I selected NMS to create a distance matrix, because NMS does not have distributional assumptions. No parasitoids emerged from two of the hot sites and four of the cold sites, so I excluded these from analyses. To select appropriate dimensionality, I used “Autopilot mode”. Then I used Sorensen’s (Bray-Curtis) similarity index and the “thorough” option in PC-ORD to create a dissimilarity matrix. I ran the model for 500 iterations and began at a random configuration.

I used a multi-response permutation procedure (MRPP) in PC-ORD to test for differences between parasitoid communities attacking *P. quercifex* at hot and cold sites and found no significant difference between groups ($A=-0.03$, $P=0.87$).

APPENDIX B: SUPPLEMENTAL MATERIAL FOR CHAPTER 3

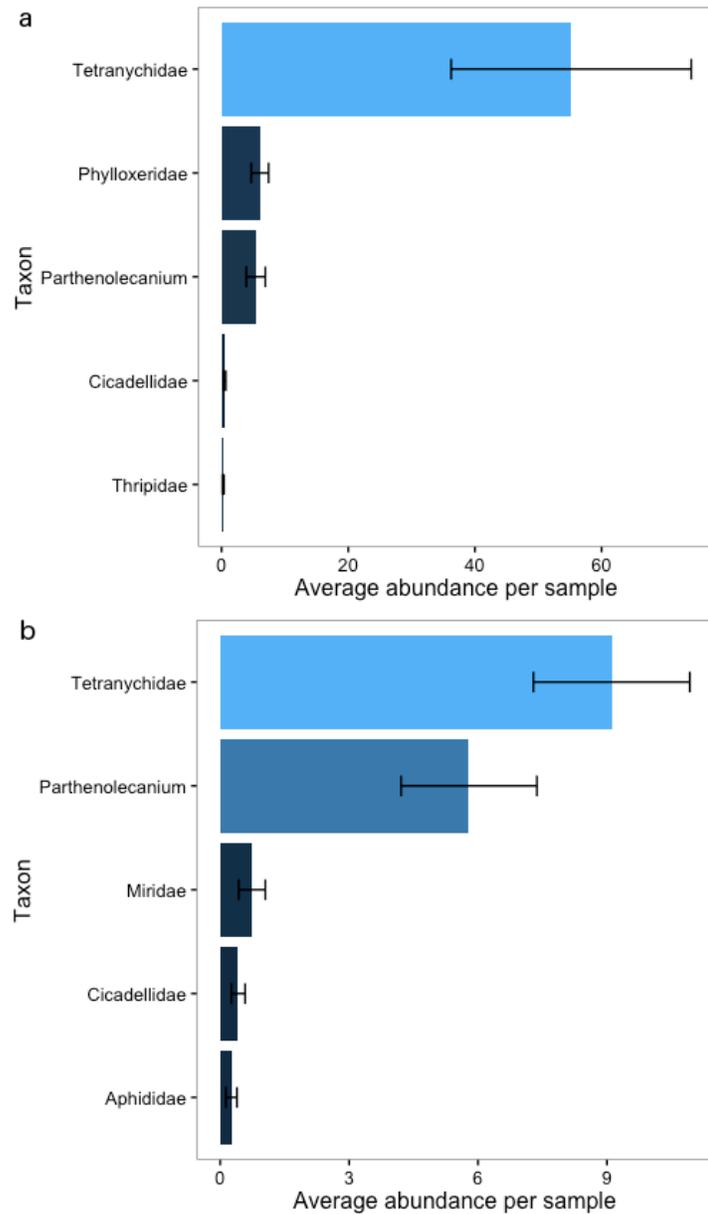


Figure S1. Spider mites (Tetranychidae) and oak lecanium scale insects (Coccidae: *Parthenolecanium* scale insects) were the most abundant herbivores on study trees. Five most common arthropods in funnel beat samples in (a) 2013 and (b) 2014. Linear models were preferred; if linear fit was poor, even after data transformation, I used generalized models. Error bars are mean±SEM.

Table S1. Detailed statistics. In all mixed effects models, "Site" was specified as the random effect. In GLMER, sample ID was also included as a random effect.

a) <i>Parthenolecanium</i> sp. abundance Aug. 2013						
	Variable	Estimate	residDF	Z	P	
Model: GLMER	Intercept	-24.92	29	-2.02	0.0437	
Error dist.: Poisson	Oil treatment (y) Mar.-Aug. 2013	-1.45		-6.40	<0.0175	
	temp.	1.62		2.10	0.0432	
b) <i>Parthenolecanium</i> sp. abundance Jun. 2014						
	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	-7.66	1	17	108.97	<0.0001
Error dist.: Gaussian	Oil treatment (y)	-0.54	1	17	18.45	0.0005
$R^2_m = 0.276$	Apr.-Jun. 2014 temp.	0.38	1	16	4.88	0.0420
$R^2_c = 0.809$						
c) <i>Parthenolecanium</i> sp. abundance Mar. 2015						
	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	-7.84	1	17	55.82	<0.0001
Error dist.: Gaussian	Oil treatment (y)	-0.32	1	17	14.33	0.0015
$R^2_m = 0.258$	Apr.-Jun. 2014 temp.	0.36	1	16	4.74	0.0049
$R^2_c = 0.790$						
d) Spider mite abundance Aug. 2013						
	Variable	Estimate	residDF	Z	P	
Model: GLMER	Intercept	-33.53	29	-2.97	0.0030	
Error dist.: Poisson	Oil treatment (y) Mar.-Aug. 2013	-1.33		-3.30	0.0009	
	temp.	2.14		3.00	0.0027	
e) A_{sat}						
	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	49.35	1	73	248.78	<0.0001
Error dist.: Gaussian	Oil treatment (y)	-0.05	1	17	0.00	0.9620
	Jul. 2014 temp.	-1.47	1	16	4.79	0.0437
f) Conductance						
	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	0.72	1	73	168.51	<0.0001
Error dist.: Gaussian	Oil treatment (y)	-0.01	1	17	0.60	0.4480
	Jul. 2014 temp.	-0.02	1	16	5.53	0.0319
g) Transpiration						
	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	11.09	1	73	193.07	<0.0001
Error dist.: Gaussian	Oil treatment (y)	-0.17	1	17	0.62	0.4406
	Jul. 2014 temp.	-0.33	1	16	4.69	0.0458

Table S1 Continued

h) Fv/Fm	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	1.50	1	73	28232.8	<0.000
Error dist.: Gaussian	Oil treatment (y)	0.01	1	17	1.04	0.3215
	Jul. 2014 temp.	-0.01	1	16	1.09	0.3113
i) Water potential	Variable	Estimate	numDF	denDF	F	P
Model: LM	Intercept	-1.49				
Error dist.: Gaussian	A _{sat}	-0.00				
Adjusted R ² = -0.027			1	36	0.03	0.8653
j) Basal area growth 2014	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	20.53	1	16	319.32	<0.0001
Error dist.: Gaussian	Oil treatment (y)	0.73	1	16	0.34	0.5659
R ² _m = 0.411	Apr.-Oct. 2014 temp.	-0.60	1	16	5.36	0.0341
R ² _c = 0.761	Original basal area	0.01	1	16	15.62	0.0011
k) Basal area growth 2015	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	4.03	1	16	3	<0.0001
Error dist.: Gaussian	Oil treatment (y)	0.01	1	16	0.01	0.9081
R ² _m = 0.291	Apr.-Oct. 2014 temp.	-0.11	1	16	5.87	0.0276
R ² _c = 0.737	Original basal area	0.00	1	16	5.15	0.0374
l) Basal area growth 2015	Variable	Estimate	numDF	denDF	F	P
Model: LM	Intercept	29.89				
Error dist.: Gaussian	A _{sat}	6.59				
Adjusted R ² = 0.079	Full model		1	34	7.99	0.0078
m) Branch growth 2014	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	1.42	1	17	9	<0.0001
Error dist.: Gaussian	Oil treatment (y)	0.07	1	17	4.60	0.0467
	Apr.-Oct. 2014 temp.	-0.01	1	16	0.07	0.7951
n) Branch growth 2015	Variable	Estimate	numDF	denDF	F	P
Model: LME	Intercept	14.21	1	17	369.84	<0.0001
Error dist.: Gaussian	Oil treatment (y)	0.31	1	17	1.28	0.2735
	Apr.-Oct. 2014 temp.	-0.45	1	16	2.47	0.1356

APPENDIX C: SUPPLEMENTAL MATERIAL FOR CHAPTER 4

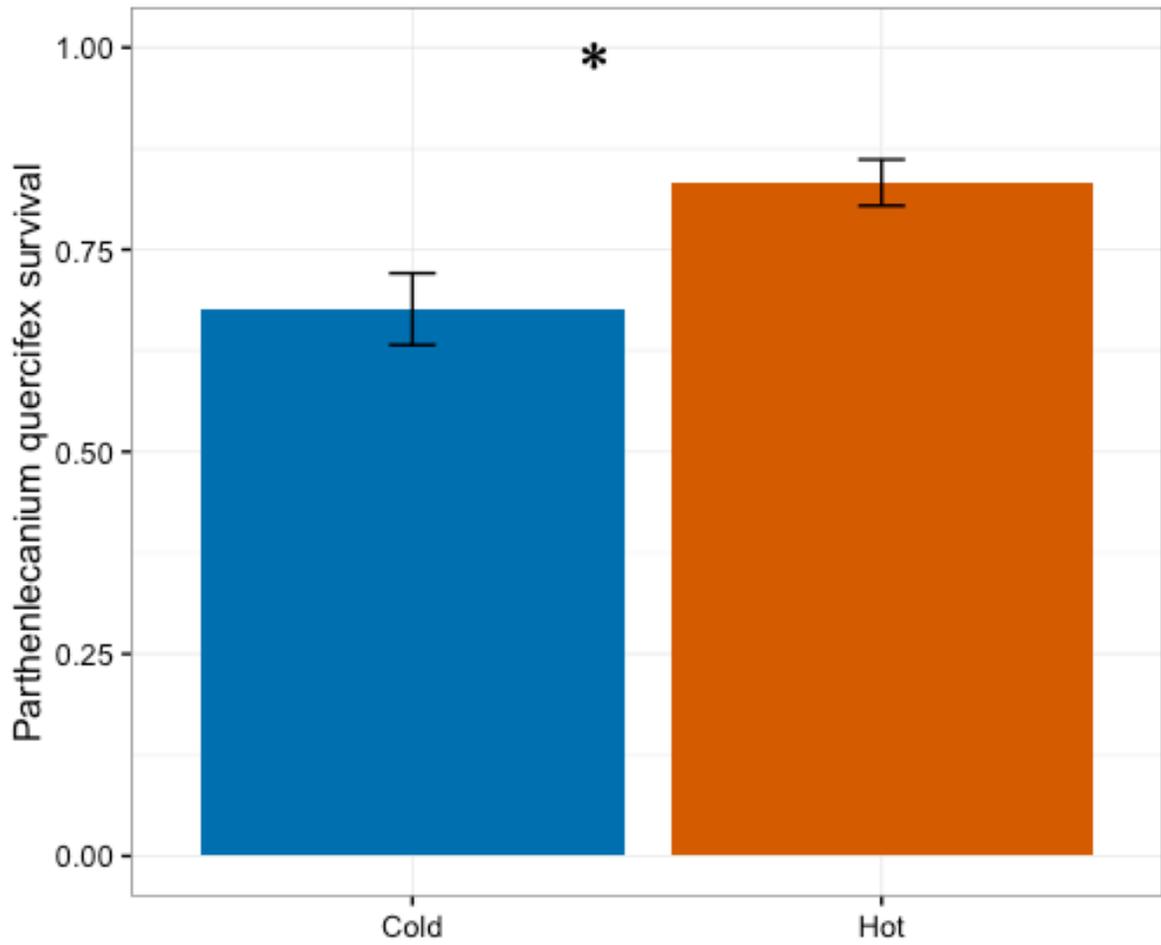
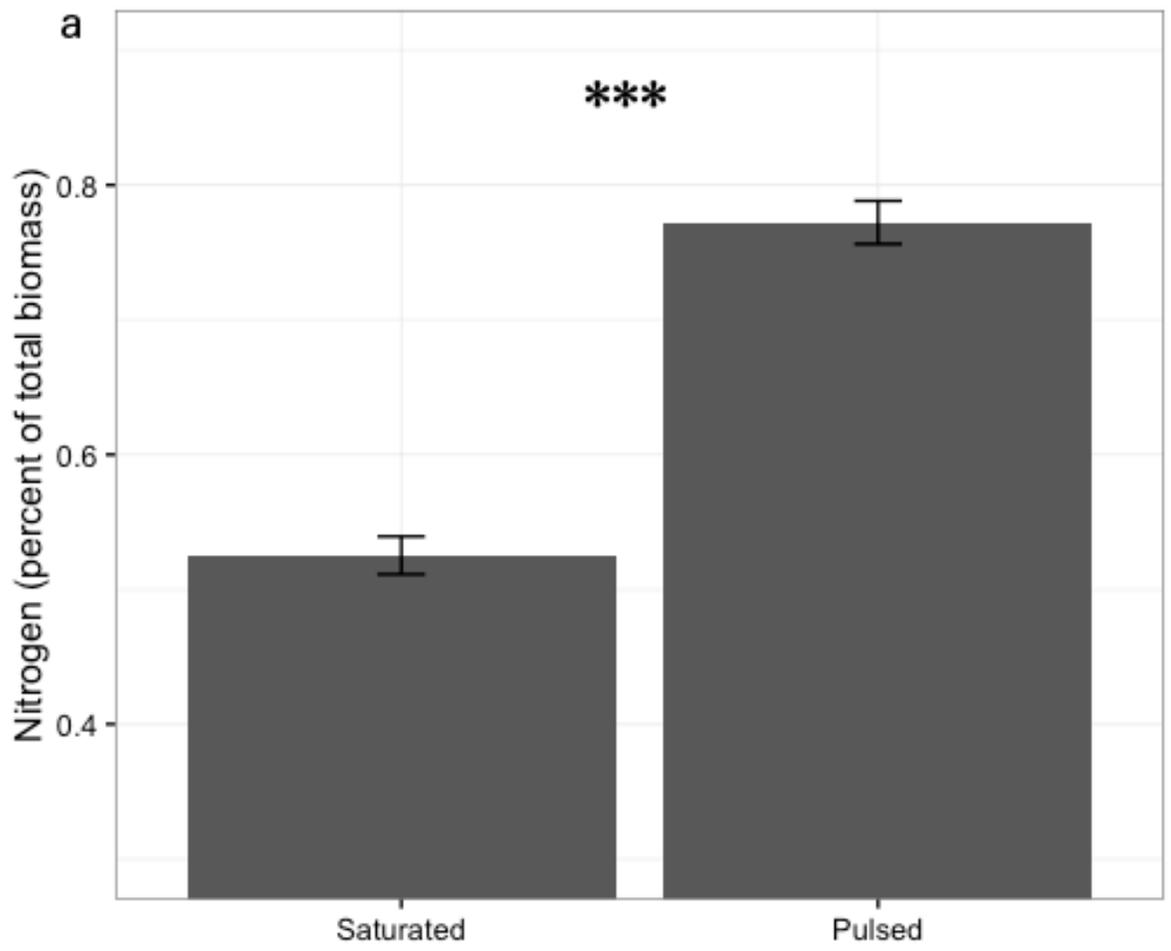
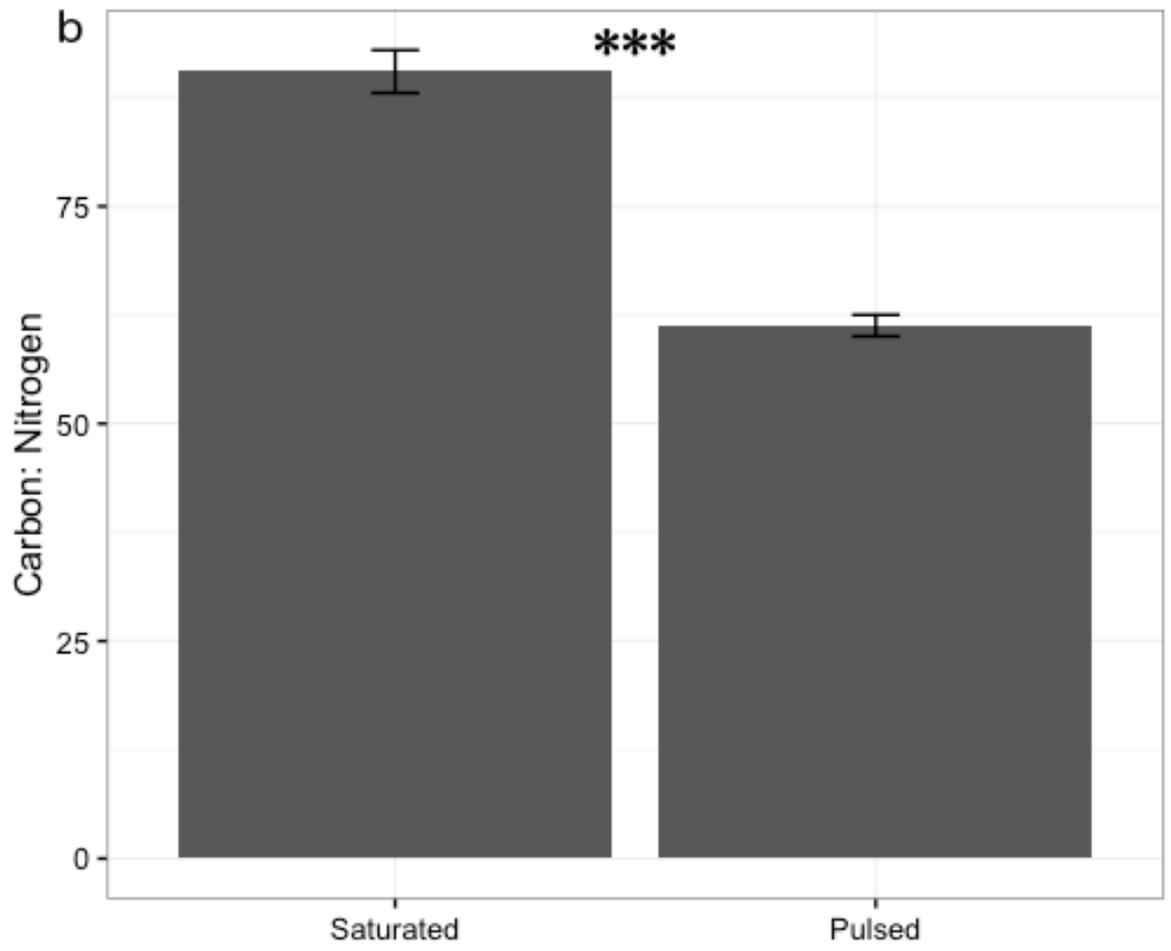
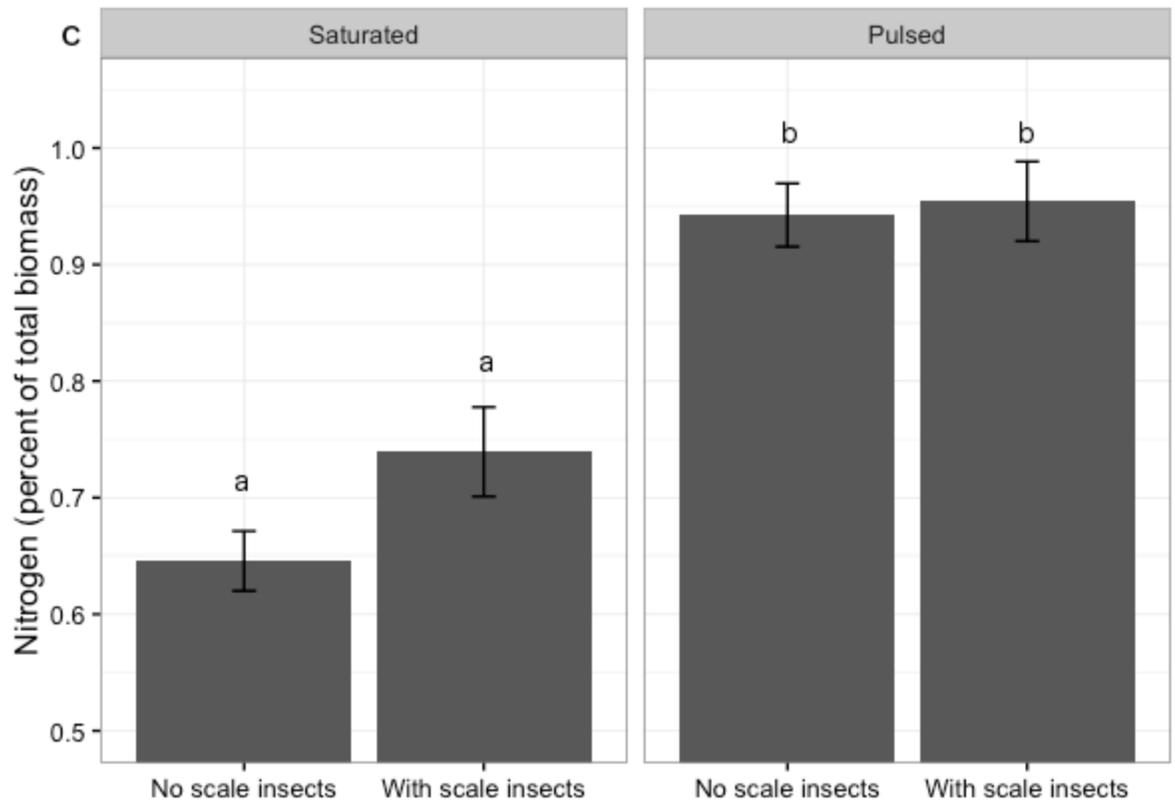


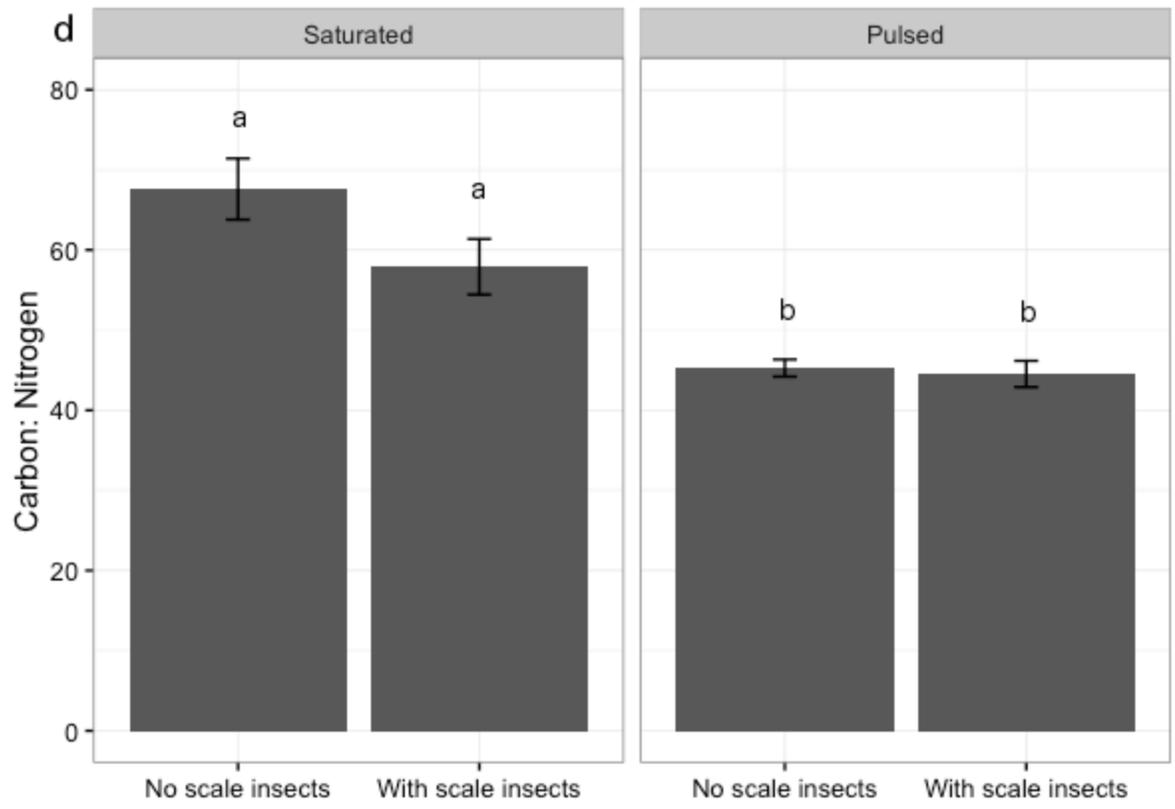
Figure S1. *Parthenolecanium quercifex* survived better in hot than in cold laboratory chambers.

Figure S2. Nutrient content of laboratory saplings at the end of the growing season. (a-b) Trees with pulsed water inputs had higher nitrogen contents and carbon to nitrogen ratios in shoots (c-d) and in roots. *Parthenolecanium quercifex* had a nearly significant effect on nitrogen contents and carbon to nitrogen ratios in roots.









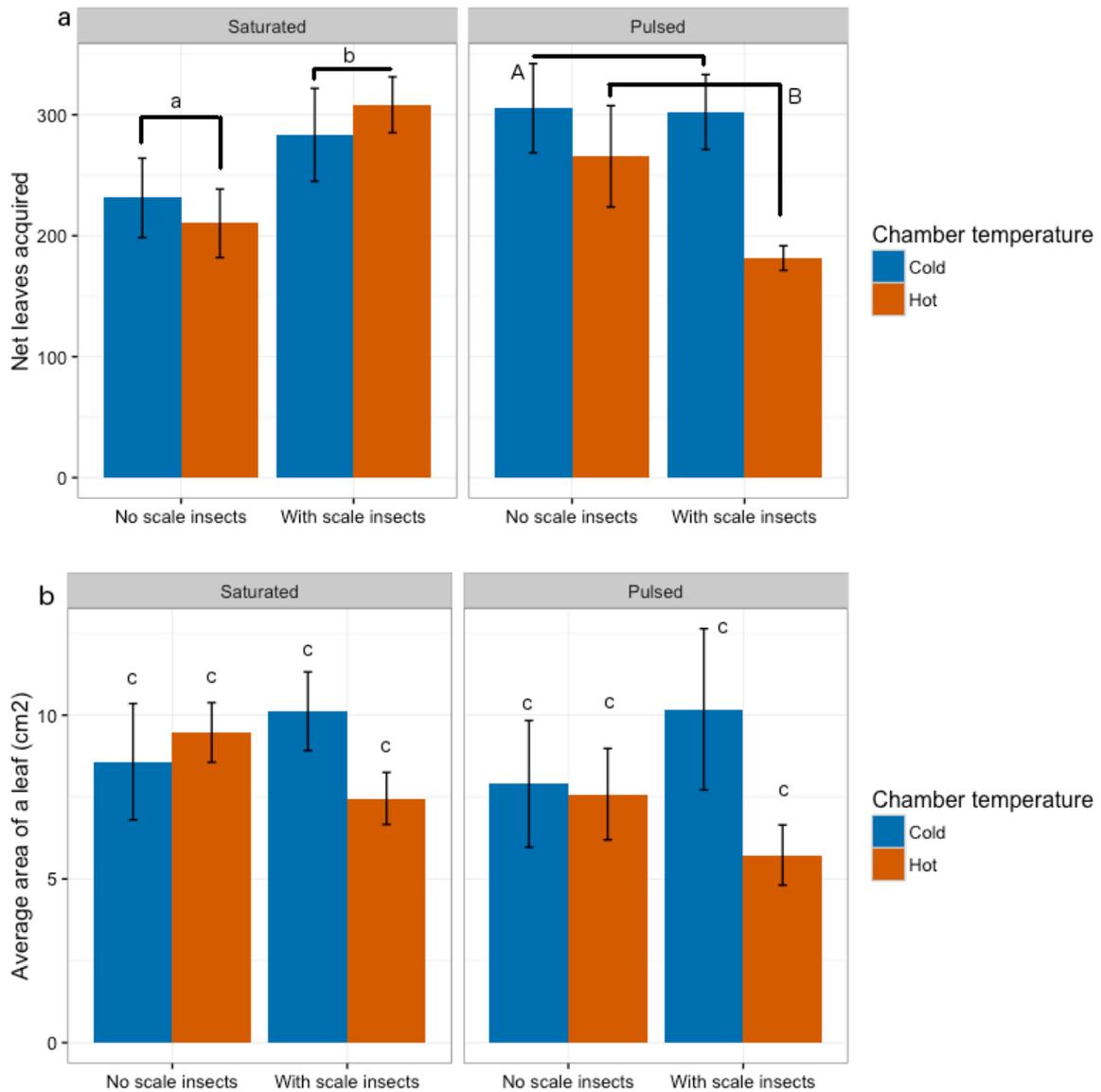


Figure S3. Effects of treatments on variables used to estimate total leaf area acquired. (a) Trees that received pulsed water inputs acquired fewer leaves but only in hot chambers. Trees with *P. quercifex* acquired more leaves than those without, but only if they were saturated. (b) There was a significant interaction between temperature and *P. quercifex*, such that leaves in hot chambers with *P. quercifex* were smaller than other treatments. All treatments are shown here to allow comparisons with figure 3a, which shows total leaf area acquired. When significant interactions were detected, letters refer to Tukey HSD tests.

Dates	Hot daytime (C)	Hot nighttime (C)	Cold daytime (C)	Cold nighttime (C)	Sunrise	Sunset	Raleigh 30-year avg. temp. normals (C)
April 5-30	21	18	18	15	525	1900	16
May	22	18	18	14	525	1900	20
June	28	24	24	20	510	1930	24
July	30	26	26	22	510	1930	26
August	28	24	24	20	510	1930	25
Sept	22	18	18	14	600	1820	22
October	20	16	16	14	720	1845	16
Nov. 1- Dec. 11	13	10	13	10	650	1710	11

Table S1. Temperatures in laboratory chamber experiment.