A Thesis

entitled

Effects of Air *vs*. Air+Soil Heating During a Simulated Heat Wave on White Oak (*Quercus alba*) and Black Oak (*Quercus velutina*)

by

Nicole E. Lightle

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Biology

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An Abstract of

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Extreme weather events are a growing focus of global climate change research. Extreme events, which occur abruptly and unpredictably, are often more detrimental to terrestrial vegetation than gradual shifts in climate. One type of event, the summer heat wave, may already be increasing in some areas of the world. Large-scale reductions in Net Primary Productivity and mortality have been reported during heat waves in forested ecosystems. Unfortunately, our understanding of how abrupt heat stress affects woody species during heat waves lags behind our knowledge of herbaceous species that have been more widely studied in experimental manipulations. A few studies of herbaceous species also suggest that the coupling of soil heating to air heating can change the overall plant response to heat waves. To investigate air *vs*. air+soil heating in woody species, we manipulated the temperature of both shoots and roots separately for both white and black oak seedlings by insulating the soil during heat-stress to the shoot (35 *vs.* 40^{o}C for 4 days, white oak; 35^{o}C for 8 days, black oak). Interestingly, at moderate heat-stress temperature (35^{o}C), net photosynthesis declined and internal CO_2 concentration of leaves increased more when the roots were insulated in both species. Hence, concurrent soil warming prevented metabolic damage to leaves during moderate heat-stress, suggesting that direct heat to the roots increased shoot thermotolerance. In both experiments, differences in air *vs*. air+soil heating effects on root respiration were directly related to differences in soil temperatures, such that root respiration was higher with air+soil heating. In neither experiment were soil temperature effects related to plant water status. These results suggest that both direct and indirect effects of soil warming may occur in woody species during a heat wave, but that the response may depend on the severity and duration of the heat-stress. Future research is needed to determine the underlying mechanism for differences between air *vs*. air+soil heating during a heat wave.

Because my happiest childhood memories were spent playing in a forest which is no longer extant, this thesis is dedicated to the Lorax and all the children who look around their world, letting it fill their head with questions, and dream of becoming scientists.

Acknowledgements

I would first and foremost like to thank my family for their continued support, which gives me the strength to continue to follow my dreams. I am proud and happy to finally be on the path to becoming a scientist; without their love and encouragement I would never had made it this far. I'd like to thank my committee members for their kind guidance and assistance during the final semester of my masters. I'd also like to thank Dr. Weintraub for allowing me to attend his lab meetings and for showing me that in many ways dirt (*i.e.* soil) can actually be very interesting! I'd like to thank all those at UT involved in the NSF GK-12 program which allowed me to experience many unique opportunities during my masters including working at Bowsher High School. I would also like to give special thanks to Mr. Bourland, who invited me into his classroom as a fellow colleague and provided me many wonderful opportunities to learn how to become a better teacher. In that regard, I would also like to acknowledge the students at Bowsher who helped motivate and inspire me on a daily basis. They showed me that a student's enthusiasm to learn can only be limited by the experiences and opportunities that are available to them. I also wanted to thank my labmates, especially Sasmitta, whose desire to show kindness to everybody and the ability to drop everything to help out another student, is truly admirable. And finally, I'd like to thank my advisor who, in knowing my love for physiology, extended me the wonderful opportunity to learn about and experience the inner workings of a tree.

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Chapter 1

Introduction

Although global climate change is predicted to increase the global mean annual surface temperature by 1.5-4.5^{o}C over the next century, both the frequency and severity of extreme climatic events are also supposed to increase (IPCC 2007). One type of extreme weather event predicted to increase is the summer heat wave. A temperature anomaly that comes on suddenly, like a heat wave, can be more detrimental to a plant than a gradual warming, because of the lack of time for plant acclimation (Rennenberg *et al*. 2006). In addition, heat waves are typically accompanied by drought (De Boeck *et al*. 2010), which may then result in additive or synergistic negative effects on plants.

The majority of plant research on acute heat-stress is still focused on small herbaceous species, rather than larger woody species, which, due to their size, makes manipulating air temperature in experimental studies with adult trees difficult. For woody species, this technical difficulty means that we have little direct evidence for how longer events of acute heat-stress is going to affect trees during heat waves. Aside from a limited number of experimental manipulations done on tree seedlings, most of our knowledge of tree response to acute heat-stress comes from a few natural events, like the 2003 heat wave in Europe. This 2003 heat wave, which spanned the entire summer, was preceded by drought and offered up temperature anomalies so severe, it was thought to provide direct evidence that a change in the frequency distribution of mean temperature was co-occurring, along with the gradual shift of the mean temperature to warmer conditions (Rebetez *et al*. 2006). A change in this frequency distribution would ultimately bring even more heat waves in the future, making impacts on terrestrial vegetation even more important to understand (Schar *et al*. 2004)

From events such as the 2003 heat wave, a combination of drought *x* heat has been shown to decrease forest Net Primary Production (NPP) and cause landscape-scale mortality in certain instances (Allen *et al*. 2010; Ciais *et al*. 2005). Proposed research to understand these events is still largely focused only on the water effect or has not been able to separate heat *vs*. drought effects, although heat in and of itself can be detrimental to trees (Hartmann 2011; McDowell *et al*. 2008). Heat-stress causes an immediate reduction in photosynthesis, increases respiration, and can cause water stress (Weis and Berry 1988). As with other abiotic stresses that occur simultaneously, individual response to heat *vs*. drought cannot be teased apart when both occur together (Mittler 2006). In the instance of the 2003 heat wave, the confounding of drought with heat does little to enhance our knowledge of how trees will react to heat alone, making more experimental manipulative studies needed.

Roots also have the potential to experience heat-stress effects, either directly through soil heating or indirectly via effects on the shoot (*e.g.*, via reduced carbon flux from shoot to roots, or increased shoot water demand). Direct heating has been imposed to soil in many manipulative studies designed to examine the thermotolerance of the root system (Graves *et al*. 1991). From these studies as well as others, we know that roots of woody species have a small temperature tolerance range, often experiencing optimal growth at around 20-30°C (Graves *et al*. 1991; Perry 1982). But these studies, which only heated the soil, are likely to give different results than heating applied to both the root and shoot together (Huang *et al*. 2012). The discrepancy in results among types of heating treatments is due to direct and immediate effects of heating on the shoot during heat-stress. In shoots, there is typically an immediate decrease in photosynthesis and increase in shoot transpiration during heat-stress, which can affect the root system indirectly. The reduction in new photosynthate decreases food supply to the root, and increased transpiration in the shoot increases the demand for water on the root system. These two factors could lead to an indirect effect of heat-stress on the root system. Although this is true, few studies have looked at indirect (shoot only) *vs*. direct (shoot + root) heating effects on the root system (Huang *et al*. 2012).

The available literature indicates that heating the soil during acute heat-stress caused by air heating is more detrimental to both shoot and root functioning than shoot heating alone (Kuroyanagi and Paulsen 1988; Talanova et al. 2003; Udomprasert et al. 1995; Xu and Huang 2000). The few past studies of this type have been conducted almost exclusively on herbaceous species, and mostly on *Agrostis* species (bentgrass) by one group (Xu and Huang 2001). In fact, the only similar study conducted on a woody species that we are aware of is a recent investigation on *Prunus mira* Koehne (smooth-pit peach), which examined shoot *vs*. shoot+root heating effects on photosynthesis (specifically photosystem II), leaf water status, anti-oxidants, and abscisic acid (ABA) levels in leaves (Hao *et al*. 2012). In this study, heat-stress was imposed for 8 hours during only 1 day, and so, it does not inform us as to the potential cumulative effects of a multiple day event, such as a naturally occurring heat wave. However, their results indicate that a woody species can exhibit a somewhat different response to soil heating than the herbaceous species examined so far (*e.g.*, increases in photosynthesis in woody species with shoot+root heating *vs*. decreases in herbaceous species). Further, the Hao *et al*. study did not monitor biomass or root function, and Huang *et al*. (2012) have shown that heat effects on roots are related mostly to effects on root carbon metabolism. Given the paucity of studies of this kind, especially in woody species, and the potential differences between woody and herbaceous species in how they are affected by heat (*e.g.*, greater likelihood of hydraulic failure in woody species); (Hartmann 2011; McDowell et al. 2008), more heat-stress research is needed in woody species if we are to understand the potential impacts of increasing heat waves on forests.

In this study, which examines the separate and combined effects of shoot and shoot+root heating on a woody species, we selected two species of *Quercus* (oak) which are common across the Eastern deciduous forests of North America. The genus *Quercus* is the largest tree genus in North America, with over 70 species in the United States (Johnson *et al*. 2009). Two oak subgenera exist: *Lepidobalanus* (the white-oak group) and *Erythrobalanus* (the red-oak group). *Quercus alba* (white oak) and *Quercus velutina* (black oak) are part of the white and red oak groups, respectively. Both species have similar ranges in North America: from southern Canada to northern Florida, and from the east coast to the eastern boarder of Kansas (Burns and Honkala 1990). The local distribution of oaks is influenced by physiography, soil moisture, and geology. *Q. alba* and *Q. velutina* are well adapted to survive in a wide range of soils and sites and are common in many areas, with *Q. alba* being slightly more tolerant of mesic sites, and *Q.*

velutina being slightly more tolerant of xeric sites (Johnson *et al*. 2009). As widely distributed trees, with similar ranges and moisture tolerances, *Q. alba* and *Q. velutina* are good model species to study effects of acute heat-stress in trees.

Chapter 2

Methods and Procedures

2.1 Plant Propagation

Quercus alba (white oak) was grown from 0-1 (1-yr-old) bare-root stock purchased from a Pennsylvania grower in May of 2011. Seedlings were planted in a peat-moss-based media (Pro-mix BX Mychorrhizae), using 20 *x* 80 cm pots to prevent pot-binding. Slow-release fertilizer (NPK + micros; Osmocote Plus Northern 15-9-12) was provided shortly after planting by top-coating the pots at the manufacturer's suggested "low fertilization rate" (4.1 kg/m^3). Trees were grown in the greenhouse at *ca.* 28/28±5°C night/day and *ca.* 520-1120 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetically active radiation (PAR), and were watered as needed to keep the soil moist. Plants were treated as needed with pesticides to control thrips, leaf-hoppers, and fungus gnats, and plants used in experiments had minimal insect damage at the start of the experiment.

Quercus velutina (black oak) was grown from seed stratified in a cold room for two months in wet sand. Acorns were planted into the same medium as above, using 5 *x* 18 cm rocket pots. Slow-release fertilizer was incorporated into the planting medium at the manufacturer's-recommended low-fertilization rate (3 g/L). Seeds were germinated in a growth chamber at 28°C/20°C day/night temperatures and at 200 $\mu mol\ m^{-2}\ s^{-1}$ PAR during a 16-hr photoperiod. Soil was kept moist throughout the germination period through the use of watering trays. After two months of growth, seedlings were transplanted to 10 *x* 35 cm pots using the same soil and fertilizer mix as stated above for germination, and then plants were moved to a growth room set to 26°C/26°C day/night temperature and 400 µmol m^{-2} s^{-1} PAR during a 16-hr photoperiod. At this time, plants were provided 300 ml of 1/3-strength modified Hoagland's nutrient solution (pH 6.2) on a weekly basis.

2.2 Preliminary Experiment

To determine the temperatures required to yield moderate or severe heat-stress in our study species, an initial experiment was conducted to determine the temperature sensitivity of photosynthesis. Because Photosystem II (PSII) is highly sensitive to heat-stress, photosynthesis was monitored by measuring PSII fluorescence at various temperatures to determine the temperature at which PSII fluorescence begins to decline (Barua and Heckathorn 2006; Wang *et al*. 2008). PSII function, as quantum yield in light-adapted leaves (F_V'/F_m'), was measured using a modulated chlorophyll fluorometer (model OS1-FL, Opti-sciences) on a newly-expanded leaf receiving full light. PSII function was measured at 28°C (*ca.* near-optimal ambient daytime growth temperatures), and then again as temperature was increased every half-hour for three hours (*i.e.*, measurements at 28, 35, 38, 41, 43, 45, and 47°C).

2.3 Main Experiment- Heat Treatments

Two separate main experiments were conducted for this investigation. An initial experiment was conducted with *Q. alba* subjected to air heating alone or air+soil heating, and in this experiment, trees were heat-stressed for 4 days at either 35 or 40°C. A second experiment was conducted with a second species (*Q. veluntina*) to compare with soil-heating effects observed in the first experiment, and to extend the duration of heat treatment; in this experiment, trees were heat-stressed at 35^{o}C, and harvested at either 4 or 8 days.

Seedlings were exposed to a 6-hr heat treatment in a controlled-environment growth chamber for 4 days in Experiment 1 (*Q. alba*) and for 8 days in Experiment 2 (*Q. velutina*). Plants in Experiment 2 were acclimated to the growth chamber for one week prior to the start of the experiment. Plants were heated at either 35 or 40^{o}C (= moderate or severe heat-stress, respectively, as determined in the preliminary experiment); controls were maintained at 28^{o}C/20^{o}C day/night temperatures for Experiment 1 and 26^{o}C/20^{o}C day/night temperatures for Experiment 2. A 14-hr photoperiod was used in both experiments, with light levels at *ca.* 800 µmol m^{-}2 s^{-1} PAR at the tops of the plants. Plants were well-watered throughout each experiment.

2.3.1 Root Insulation

To investigate effects of air heating vs. air+soil heating and if there are indirect effects of heating on roots (*i.e.*, effects of air heating on roots when soil heating does not occur), we compared plants which had roots insulated to limit soil warming during heat-stress to those which did not. Insulating root boxes were constructed from closed-cell foam (FOMULAR 250 rigid-foam exterior-wall sheathing); these boxes fully-enclosed the pots, the tops of the foam boxes were flush with the tops of pots, and a layer of foam was placed on top of soil within each pot (Figures 2-1 & 2-2).

Figure 2-1: In Experiment 1, *Quercus alba* seedlings were subjected to air heating alone (left) or air+soil heating (right) during a 6-hour daily heat-stress for 4 days at either moderate (35^{o}C) or severe (40^{o}C) heat-stress level.

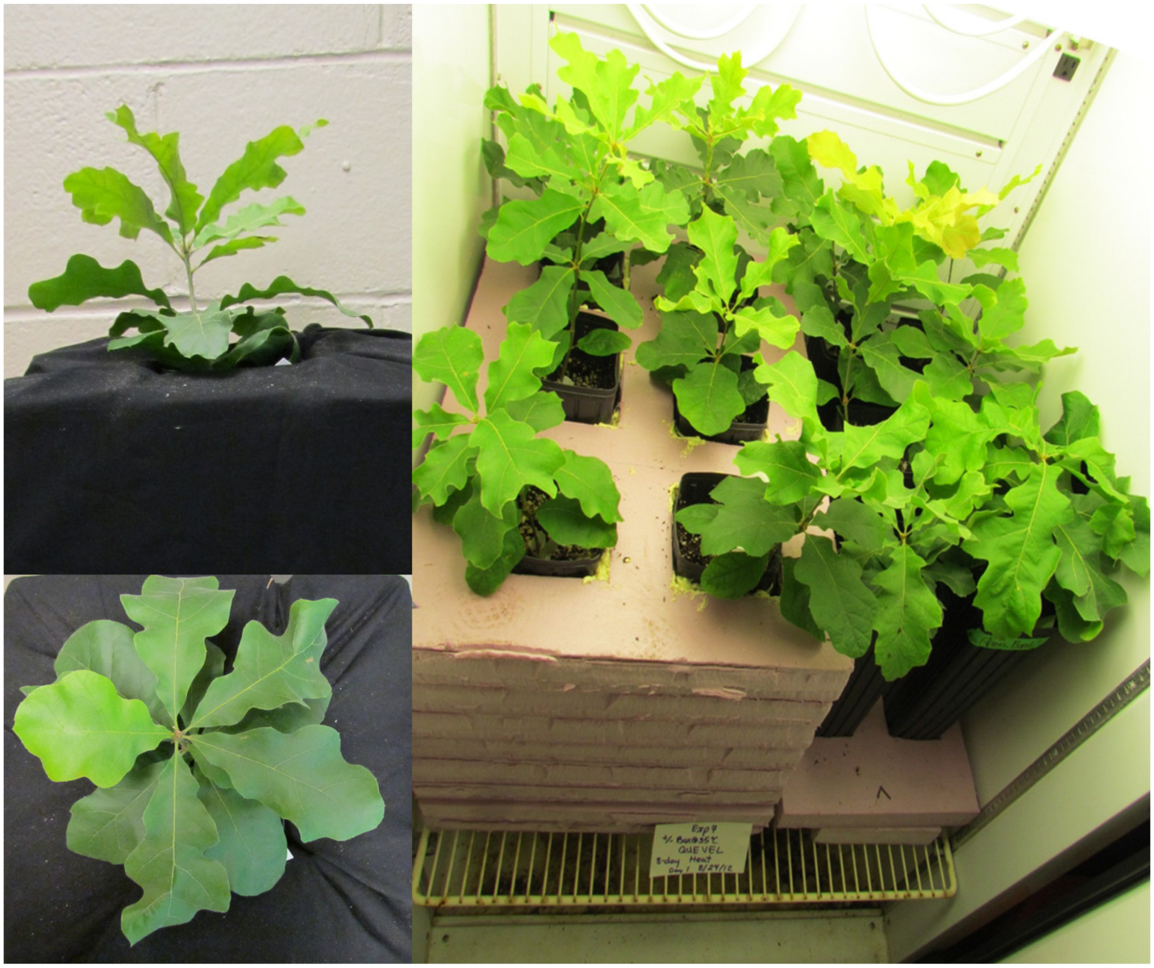
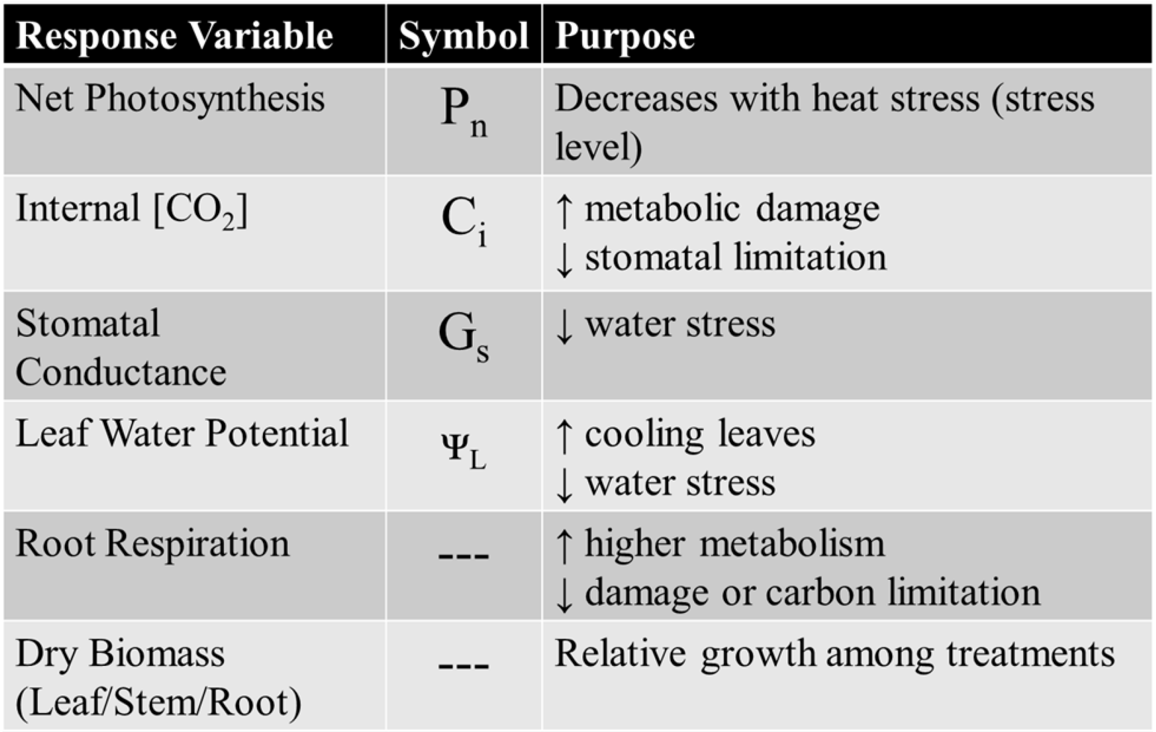


Figure 2-2: In Experiment 2, 5-½ month-old *Quercus veluntina* seedlings (top and bottom left) were subjected to air heating alone (left) or air+soil heating (right) during a 6-hour daily heat-stress for 8 days at moderate heat-stress level (35^{o}C).

2.4 Measurements

Table 2.1: Response variables measured in Experiment 1 and 2, with associated symbols and purpose of measurement.



Response Variable	Symbol	Purpose
Net Photosynthesis	P_n	Decreases with heat stress (stress level)
Internal $[CO_2]$	C_i	↑ metabolic damage ↓ stomatal limitation
Stomatal Conductance	G_s	↓ water stress
Leaf Water Potential	Ψ_L	↑ cooling leaves ↓ water stress
Root Respiration	---	↑ higher metabolism ↓ damage or carbon limitation
Dry Biomass (Leaf/Stem/Root)	---	Relative growth among treatments

2.4.1 Gas Exchange

Leaf-level gas exchange measurements were made on illuminated fully-expanded leaves using an infrared-gas analyzer-based system (IRGA) (Li-6400, LICOR, Lincoln, NE, USA), including photosynthesis (P_n; net CO_2 exchange), stomatal conductance to water vapor (G_s), and internal CO_2 concentration (C_i) (as in Barua and Heckathorn 2006; Wang *et al*. 2008). Gas exchange measurements were made with cuvette conditions at 1500 µmol m^{-2} s^{-1} PAR for Experiment 1 and 1,250 µmol m^{-2} s^{-1} for Experiment 2,370

μmol mol^{-1} CO$_2$, and at respective treatment temperatures, starting 4-hrs after the heat-stress began (and completed within 2-hrs) on one leaf per plant in all treatment plants.

2.4.2 Leaf Water Potential

Leaf water potential (Ψ_L) was measured on fully-expanded illuminated leaves at the end of the heat-stress period on one leaf per plant in all treatments using a pressure chamber (Model 600, PMS Instruments Co., Corvallis, Oregon) (as in Barua and Heckathorn 2006; Wang *et al*. 2008).

2.4.3 Soil Temperature

Soil temperature was measured at two depths (5 and 20 cm) in each pot in both the morning and evening, before and after heat-stress treatments, using a standard soil thermometer.

2.4.4 Biomass

On day 5 of Experiment 1 and days 5 and 9 of Experiment 2, plants were harvested and separated into leaves, stem, tap-root, and fine root tissue. Leaf disks were also harvested from fully-expanded leaves at this time for calculation of specific leaf mass and total leaf area. All tissue samples were oven dried for at least 48-hrs at 70^{o}C before weighing.

2.4.5 Root Respiration

Root respiration was measured during each harvest by collecting four similarly-sized non-woody root samples per plant. Detached root samples were then incubated in a 50-ml plastic tube sealed with parafilm and tape. Tubes were kept at room temperature (18-20^{o}C) or at 35^{o}C for at least 4-hrs before CO$_2$ measurement. The concentration of CO$_2$ in the tubes was measured by withdrawing a sub-sample with a syringe and injecting it into the IRGA. Respiration rates (mass based) were calculated after correcting for initial ambient CO_2 in the tubes at the time of sealing.

2.5 Statistical Analysis

Analysis of Variance (ANOVA) was performed, followed by Holm's Sidak method for multiple comparisons as a post-hoc test where appropriate (SigmaPlot 12.0 software). A *P*-value of 0.05 or less was considered statistically significant (values bolded in figures) and values between 0.05 and 0.1 are reported as marginally significant. For Experiment 1, gas exchange measurements, Ψ_L, and soil temperature were analyzed using a two-way ANOVA with temperature and root box as the main factors. Biomass and root respiration was analyzed using a one-way ANOVA with treatment analyzed as the main factor. For Experiment 2, all data was analyzed using a three-way ANOVA, with temperature, root box, and day being the main factors.

Chapter 3

Results

3.1 Experiment 1 (*Quercus alba*)

3.1.1 Soil Temperature

In experiment 1, soil temperatures (of both controls and heated plants) were lower in the plants placed in root insulation boxes, and the soil temperature in the heated plants was maintained close to that of controls, both at 5 and 20-cm depth (Figure 3-1). Maximum soil temperature (T_{max}) in un-insulated soil was 30°C (+5-6°C above controls) for the moderate heat-stress treatment (35°C) and T_{max} = 34°C (+8-10°C above controls) for the severe treatment (40°C). Soil temperature returned to control values (*ca.* 23°C) each morning (data not shown), so that similar patterns in soil temperature were observed on the first and last days of the experiment.

3.1.2 Gas Exchange

Overall, net photosynthesis (P_n) and stomatal conductance (G_s) were lowered with heat-stress treatment, regardless of heat-stress temperature (Figure 3-2). At 35°C (moderate heat stress), P_n in plants in insulated root boxes was 57% lower than in plants with warmed soils, which were similar to controls. This pattern was reversed for internal CO_2 concentration (C_i), which increased when soil temperature was lower, indicating that

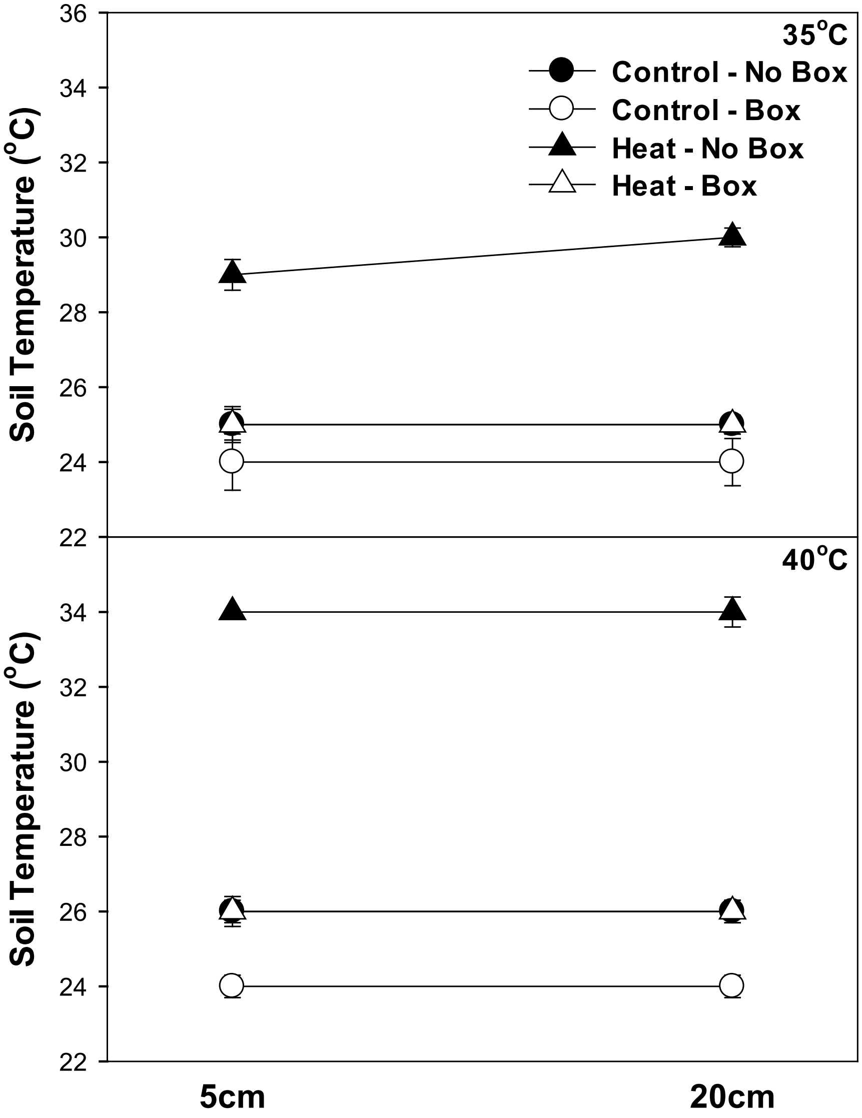


Figure 3-1: Mean ±1 SE (n=4) soil temperature (5- and 20-cm depth) of *Q. alba* after six hours of heating at 35°C or 40°C for heat-stress plants, controls were maintained at 28°C. Pots were placed in insulating root boxes (shoot heating only) or not (shoot+root heating).

the decrease in P_n in heated plants with insulated roots was not caused by the decrease in G_s. At 40°C (severe heat stress), P_n, G_s, and C_i all decreased significantly with heat, but were similar between soil temperature treatment groups. Decreases in C_i at 40°C indicated an increase in stomatal limitation to P_n.

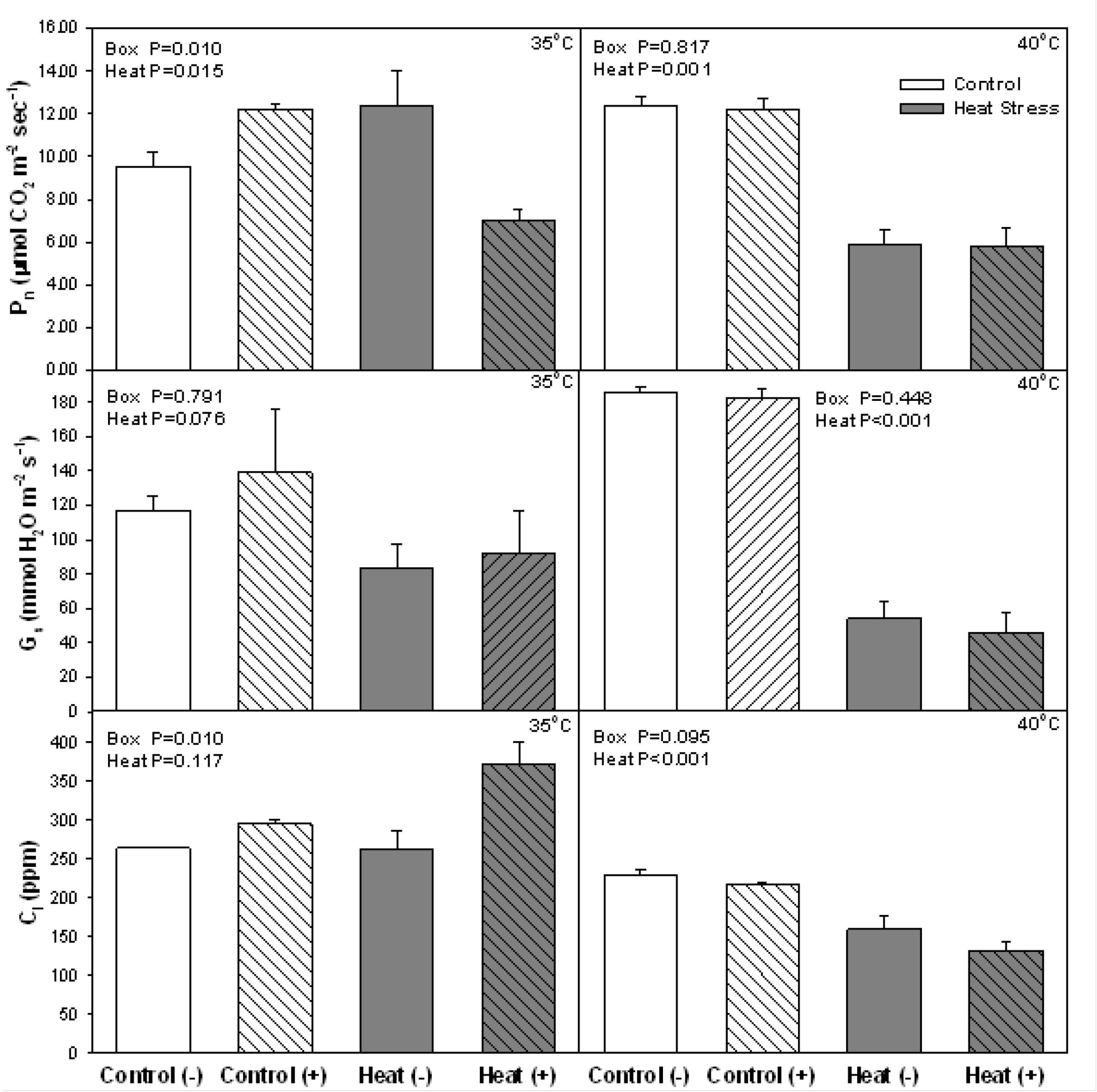


Figure 3-2: Effects of a 4-day heat-stress treatment on net photosynthesis (P_n), stomatal conductance (G_s), and internal CO_2 concentration (C_i) of *Q.alba*. Pots were placed in insulating root boxes (+) or not (-) and subjected to either 28/20°C day/night temperatures (control) or 35/40°C day (heat-stress) and 20°C night temperatures. Results are means ±1 SE, n=3-4. ANOVA results for main factors within each panel are shown.

3.1.3 Leaf Water Potential

Leaf water potential (Ψ_L) declined significantly with heat at 35°C, but heated plants did not differ from controls during the 40°C heat-stress (Figure 3-3), indicating that

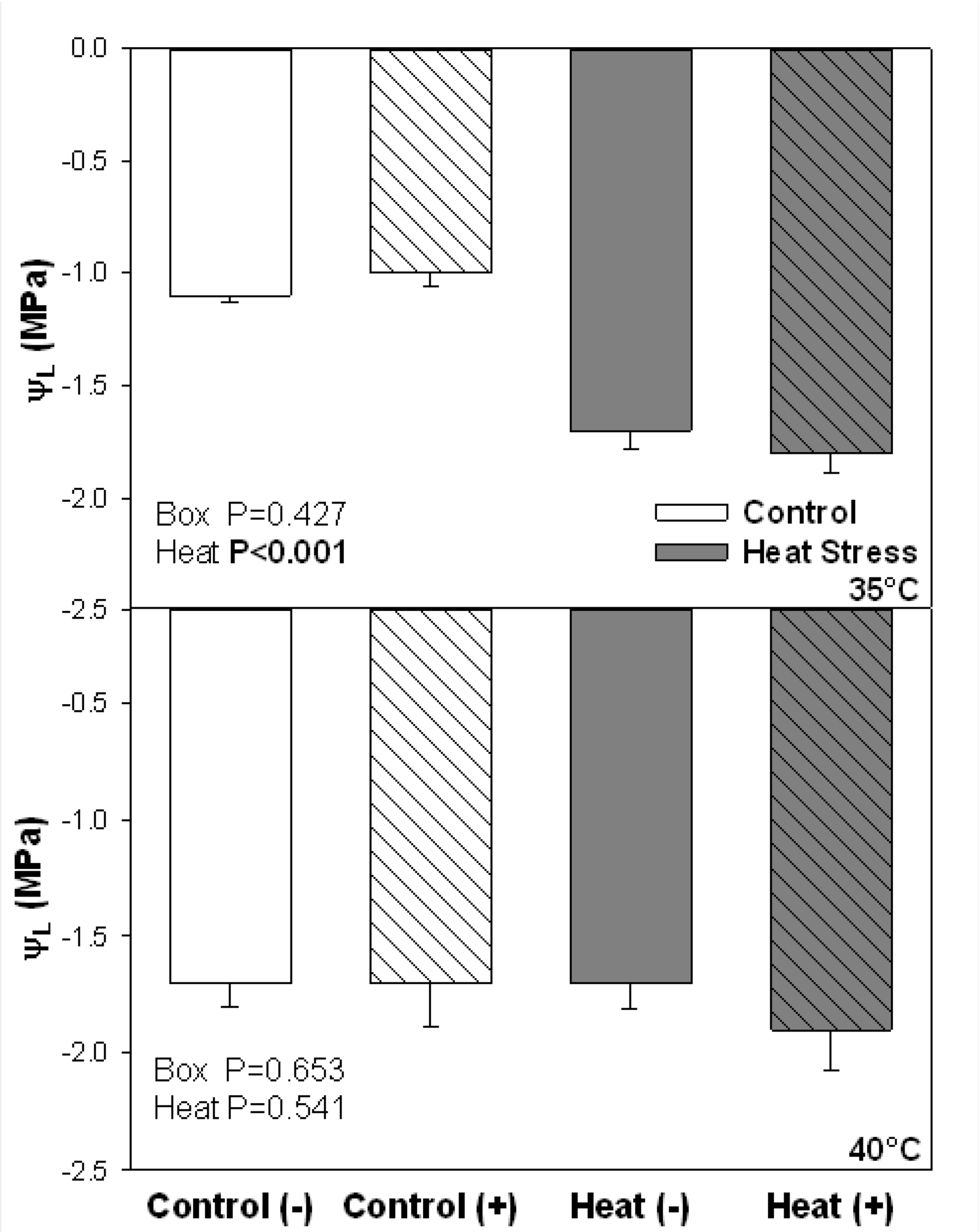


Figure 3-3: Effects of a 4-day heat-stress treatment on leaf water potential (ψ_L) of *Q. alba.* Pots were placed in insulating root boxes (+) or not (-) and subjected to either 28/20°C day/night temperatures (control) or 35/40°C day (heat-stress) and 20°C night temperatures. Results are means ±1 SE, n=4. ANOVA results for main factors within each panel are shown.

the larger decrease in G_s at 40°C (*vs*. 35°C) was sufficient to prevent declines in Ψ_L during heating, but not at 35°C.

3.1.4 Root Respiration

Root respiration rate in roots incubated at 20°C did not differ significantly between heated and control plants, indicating that no damage to respiration occurred with heating (Figure 3-4). In general, root respiration increased slightly in roots incubated at 20 *vs*. 35°C, indicating a relatively small effect of temperature on root respiration. However, incubation at 35°C caused an increase in respiration rate that was 3 times higher in plants heat-stressed at severe temperatures (40°C) in un-insulated pots than controls, and this incubation temperature (35°C) was close to that of the soil temperature (T_{max}= 34°C) experienced by these plants during the experiment.

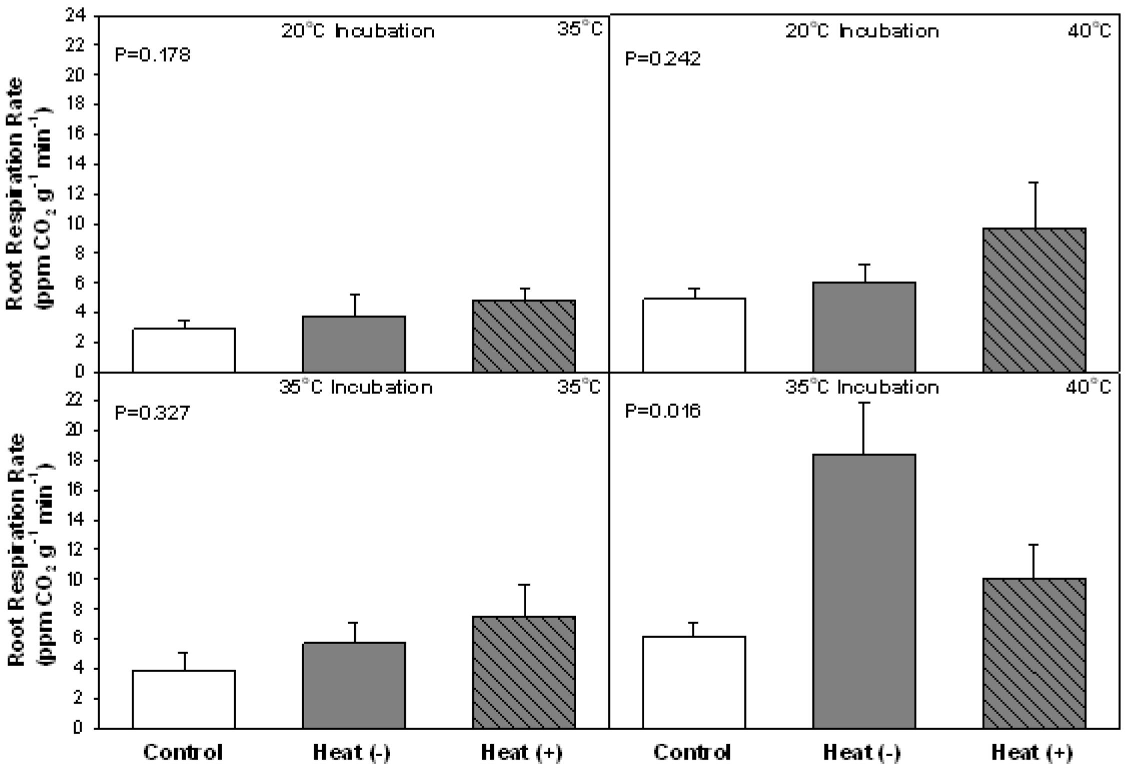


Figure 3-4: Effects of a 4-day heat-stress treatment on respiration rate of *Q. alba* roots incubated at two temperatures (20 and 35°C). Pots were placed in insulating root boxes (+) or not (-) and subjected to a 35/40°C day (heat-stress) and 20°C night; controls were kept in the greenhouse at 28/28°C day/night. Results are means ±1 SE, n=4. ANOVA results for main factors within each panel are shown.

3.1.5 Biomass

Total leaf and fine root biomass did not change significantly with heat treatment (Figure 3-5), though leaf biomass tended to decrease at 40°C in warmed soils, while fine root biomass tended to decrease at 40°C at both high and low soil temperature. Total leaf area and specific leaf mass also did not change significantly with heat treatment (Figure 3-6), although leaf area tended to decrease with heating, especially in heat-stressed plants with un-insulated pots.

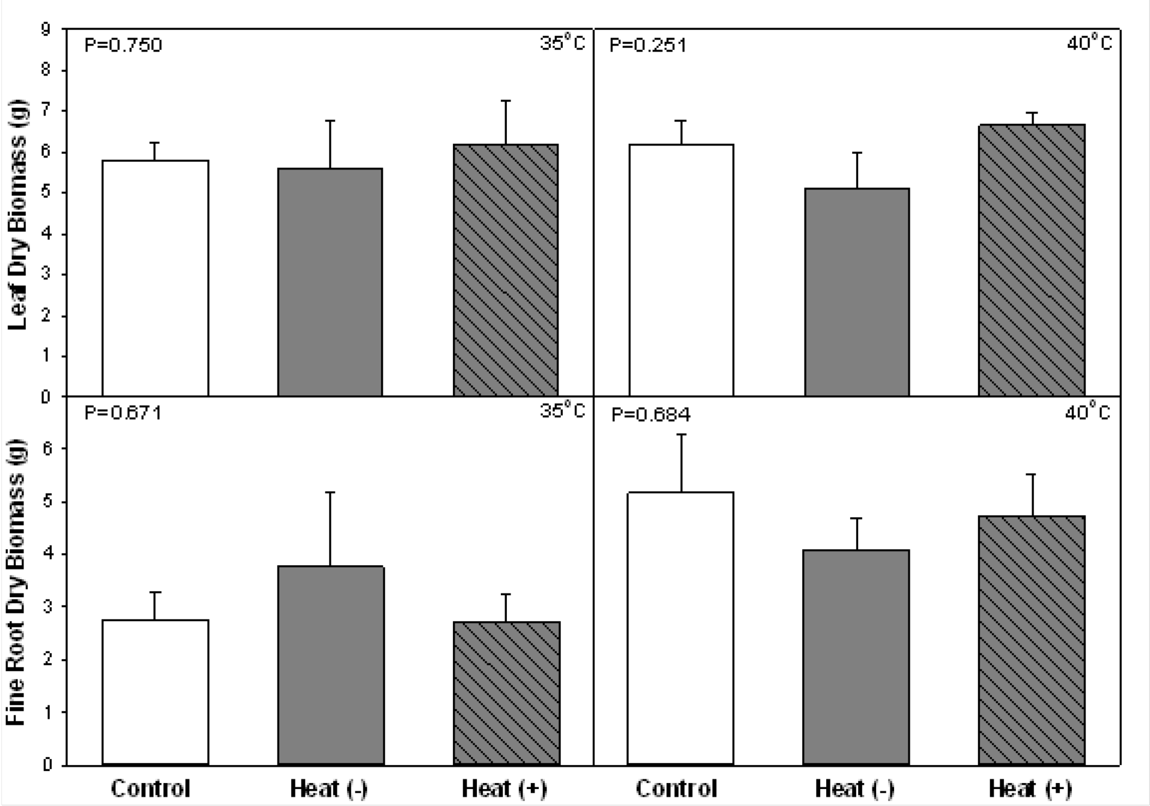


Figure 3-5: Effects of a 4-day heat-stress treatment on *Q. alba* leaf (top) and root mass (bottom). Pots were placed in insulating root boxes (+) or not (-) and subjected to a 35/40°C day (heat-stress) and 20°C night; controls were kept in the greenhouse at 28/28°C day/night. Results are means ±1 SE, n=4. ANOVA results for main factors within each panel are shown.

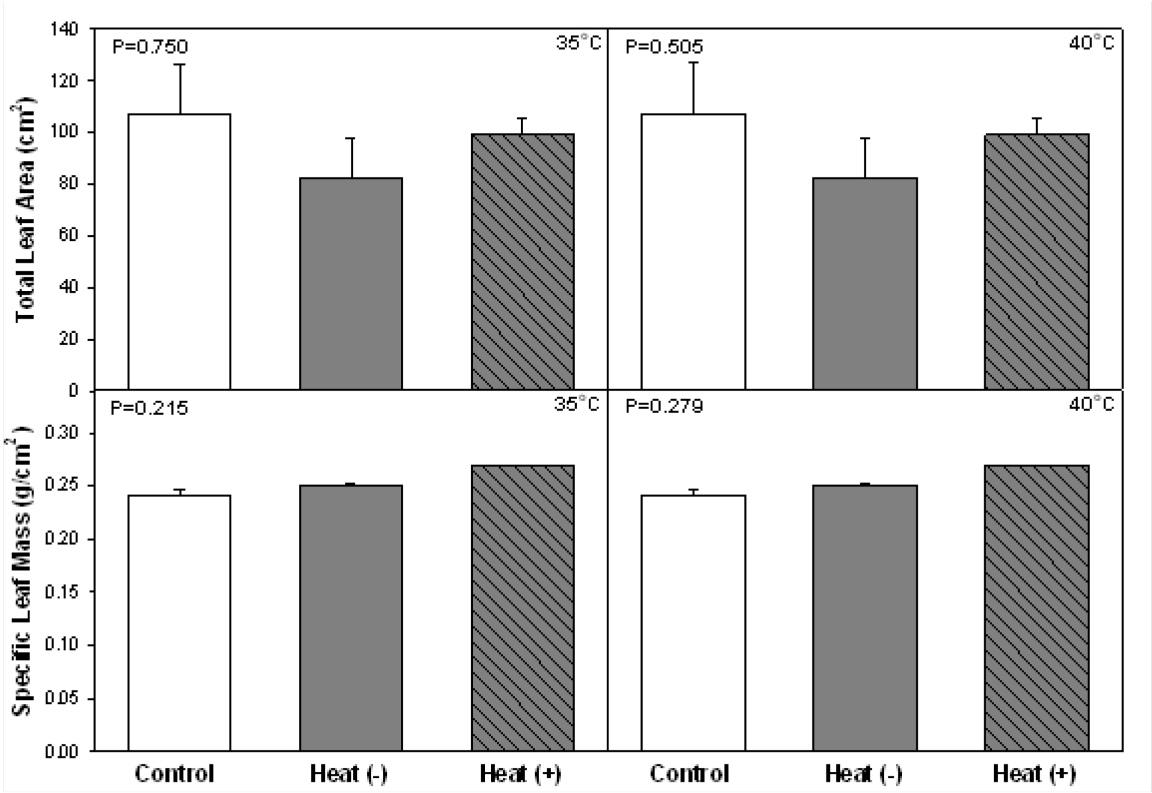


Figure 3-6: Effects of 4-day heat-stress treatment on *Q. alba* leaf area (top) and specific leaf mass (bottom). Pots were placed in insulating root boxes (+) or not (-) and subjected to a 35/40°C day (heat-stress) and 20°C night; controls were kept in the greenhouse at 28/28°C day/night. Results are means ±1 SE, n=4. ANOVA results for main factors within each panel are shown.

3.2 Experiment 2 (*Quercus velutina*)

3.2.1 Soil Temperature

In experiment 2, soil temperatures of heated plants were lower in the plants placed in boxes, with insulation maintaining soil temperature at control levels at 20-cm, but not at 5-cm depth; (T_{max} = 29°C, +6-8°C above controls) for heat-stress plants (Figure 3-7). Soil temperature in un-insulated pots (no box) returned to control values each morning (*ca.* 22°C) (data not shown), resulting in similar patterns in soil temperature being observed on days 1, 4, and 8.

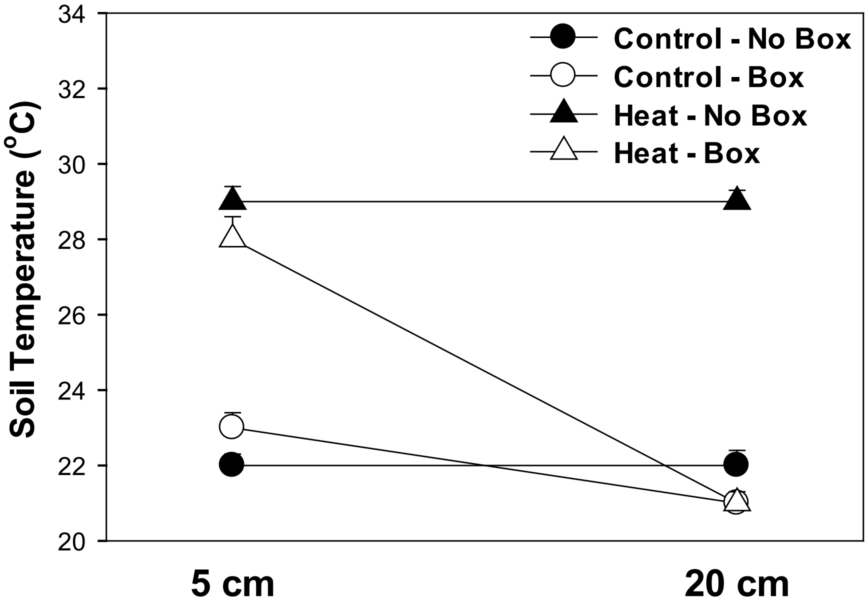


Figure 3-7: Soil temperature (5- and 20-cm depth) of *Q. velutina* after six hours of heating at 35°C for heat stress plants, while unheated controls were maintained at 26°C. Pots were placed in insulating root boxes (shoot heating only) or not (shoot+root heating). Results are means ±1 SE, n=6.

3.2.2 Gas Exchange

Both heat and root insulation decreased P_n throughout the experiment, so that heat-stressed plants in insulated root boxes had a 55% reduction in P_n as compared to warmed soils in the un-insulated (no-box) treatment group (Figure 3-8). Stomatal conductance (G_s) was also lowered by heat, while a box effect (negative) was only seen in heat-stressed plants. Both heat and root insulation increased C_i, indicating that heat- and box-related decreases in P_n were not caused by stomatal closure, but rather by effects on photosynthetic metabolism.

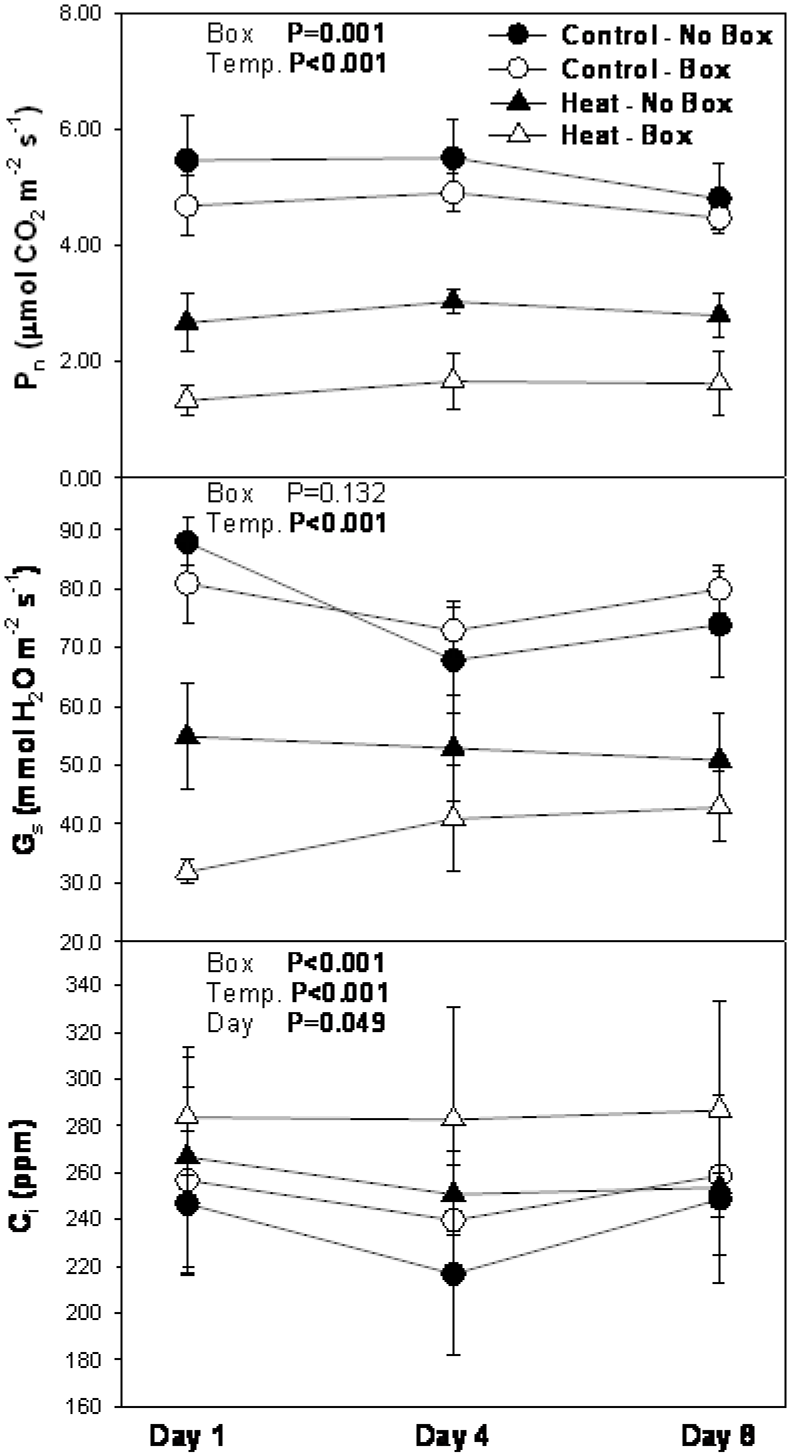


Figure 3-8: Effects of an 8-day heat-stress treatment on net photosynthesis (P_n), stomatal conductance (G_s), and internal CO_2 concentration (C_i) of *Q. velutina*. Pots were placed in insulating root boxes or not and subjected to either 26/20°C day/night (control) or 35/20°C (heat) temperatures. Results are means ±1 SE, n=6. ANOVA results for main factors within each panel are shown.

3.2.3 Leaf Water Potential

Neither heat nor soil temperature had a significant effect on Ψ_L, though there was a trend of decreasing Ψ_L in heated plants as the experiment progressed, such that by day 8, Ψ_L was slightly lower in heated *vs*. control plants (Figure 3-9).

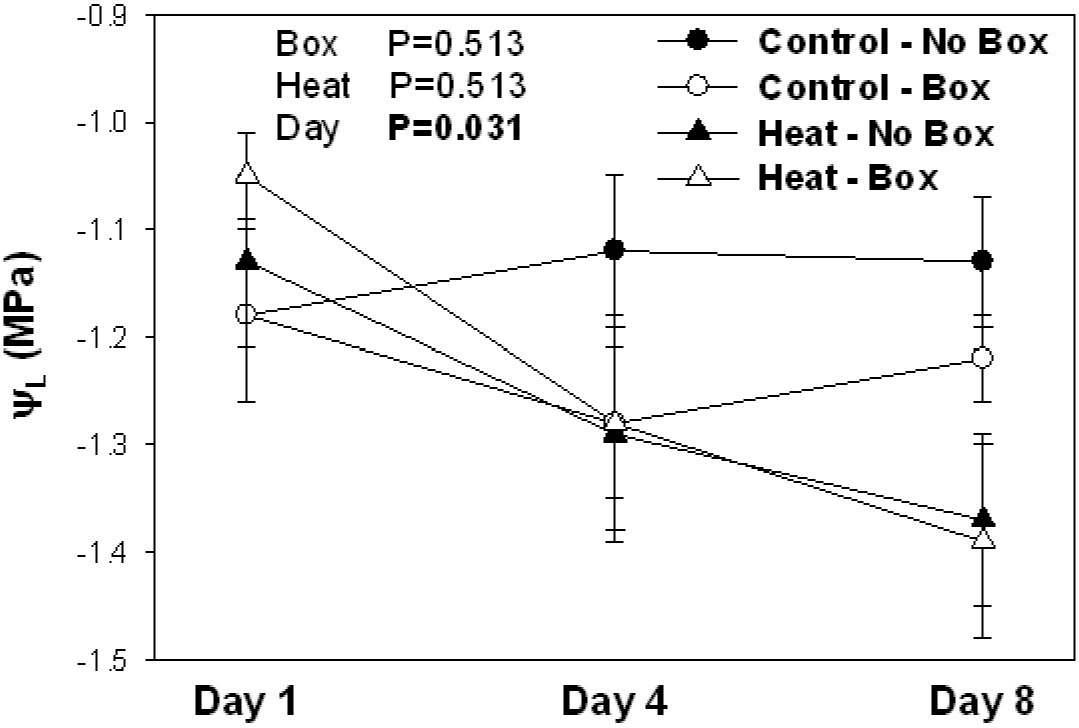


Figure 3-9: Effects of an 8-day heat-stress treatment on leaf water potential (ψ_L) of *Q. velutina*. Pots were placed in insulating root boxes or not and subjected to either 26/20°C day/night (control) or 35/20°C (heat) temperatures. Results are means ±1 SE, n=6. ANOVA results for main factors are shown.

3.2.4 Root Respiration

At both 18 and 35°C incubation temperatures, root respiration rate was higher in heated *vs*. control plants on day 8. In contrast, on day 4, respiration was slightly higher in heated plants with un-insulated pots compared to the other treatments (Figure 3-10). Respiration increased slightly with incubation temperature in all treatments.

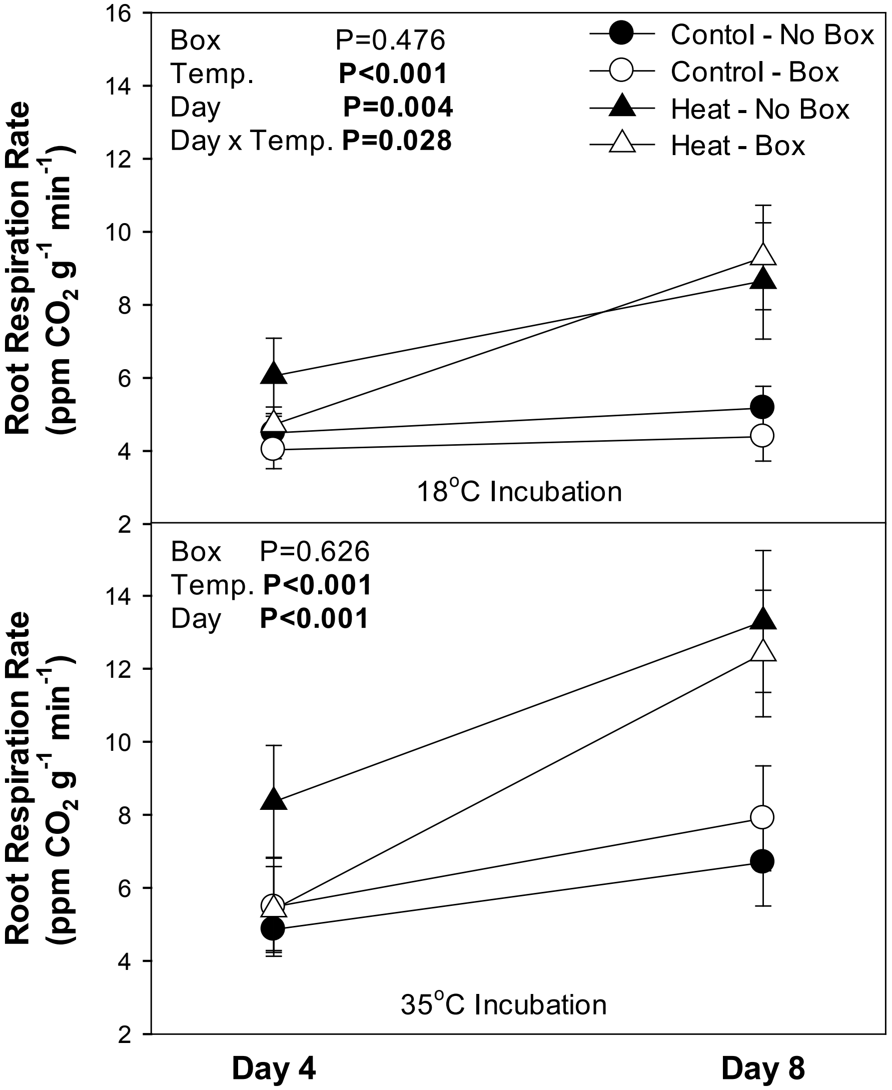


Figure 3-10: Effects of an 8-day heat-stress treatment on respiration rate of *Q. velutina* roots incubated at two temperatures (18 and 35°C). Pots were placed in insulating root boxes or not and subjected to either 26/20°C day/night (control) or 35/20°C (heat) temperatures. Results are means ±1 SE, n=5-6. ANOVA results for main factors within each panel are shown.

3.2.5 Biomass

Leaf biomass was significantly lower in heated plants, especially on day 8 in insulated plants (heat *vs*. control) (1-way ANOVA within insulated plants on day 8 only, $P<0.001$) (Figure 3-11). There were no significant overall treatment effects on total leaf area among the treatment groups; however, total leaf area was lower in heated *vs*. control insulated plants (2-way ANOVA within insulated plants only; P=0.009, day x temp. P=0.025). There were no significant overall treatment effects on fine-root biomass

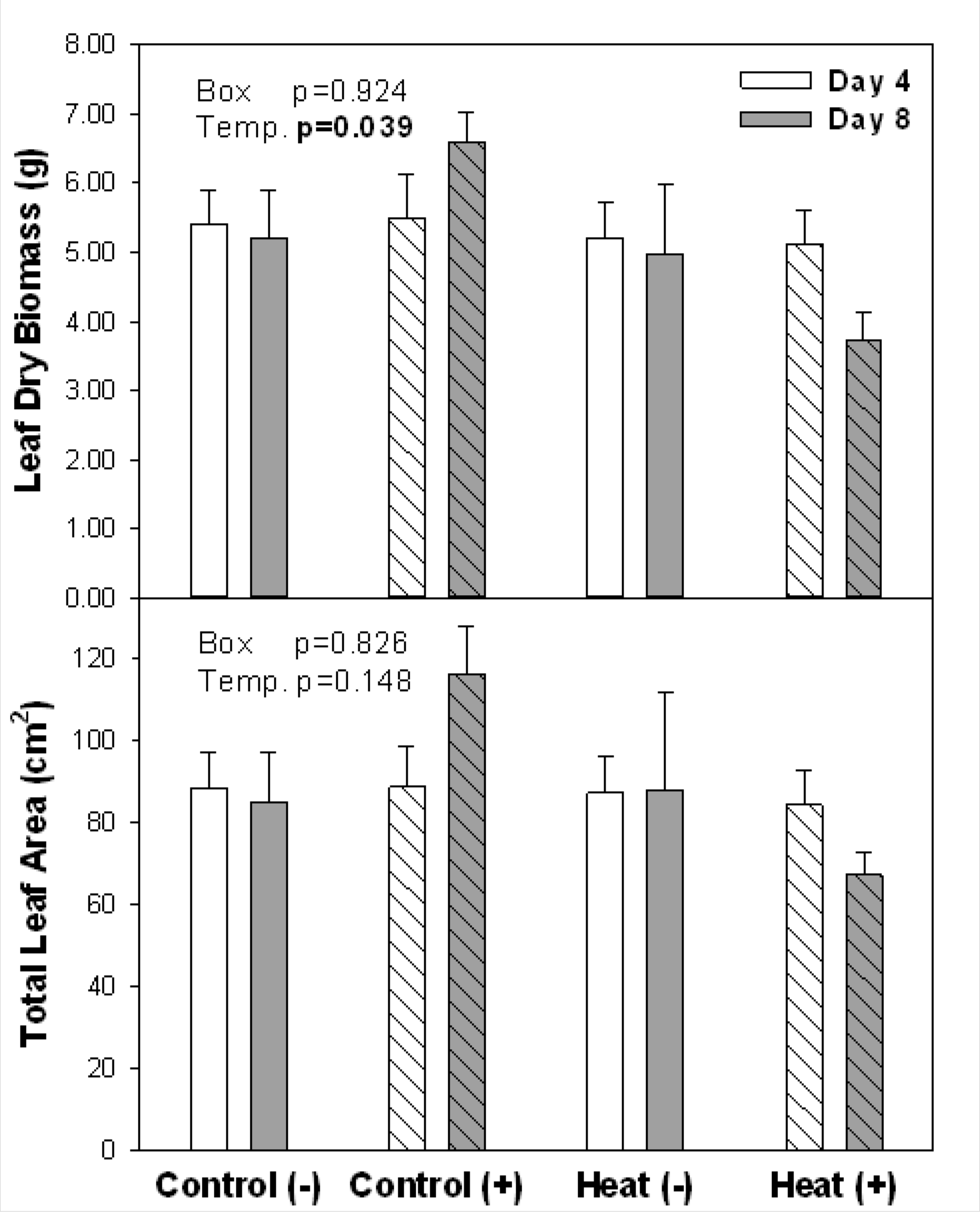


Figure 3-11: Effects of an 8-day heat-stress treatment on leaf mass and area of *Q. velutina*. Pots were placed in insulating root boxes (+) or not (-) and subjected to either 26/20^{o}C day/night (control) or 35/20^{o}C (heat) temperatures. Results are means ±1 SE, n=6. ANOVA results for main factors within each panel are shown.

among the treatment groups; however, root insulation had a marginally significant effect on heated plants (2-way ANOVA within heated plants only; P=0.065) (Figure 3-12). Tap-root biomass was marginally lower for heat-stressed plants, and this difference was similar for both insulated and un-insulated pots on both days.

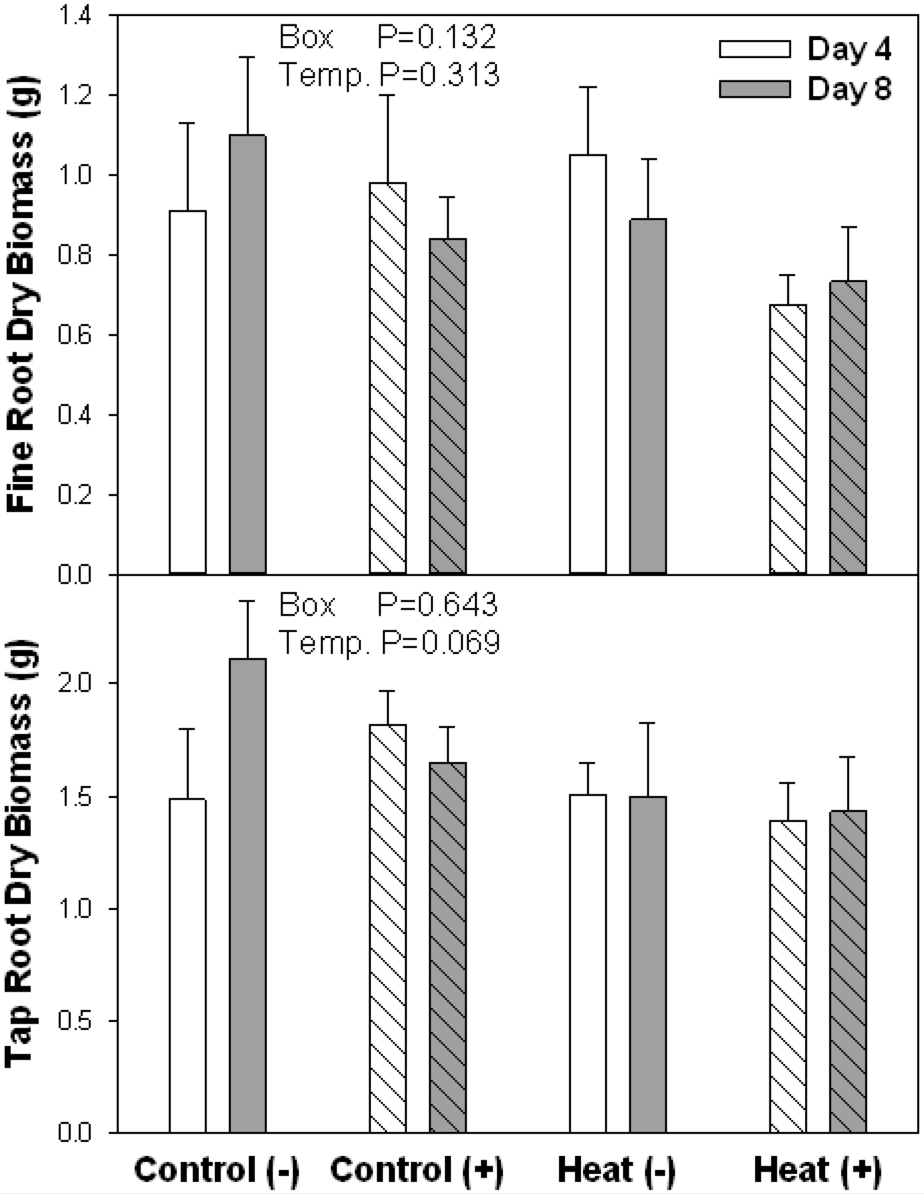


Figure 3-12: Effects of an 8-day heat-stress treatment on root mass of *Q. veluntina*. Pots were placed in insulating root boxes (+) or not (-) and subjected to either 26/20^{o}C day/night (control) or 35/20^{o}C (heat) temperatures. Results are means ±1 SE, n=6. ANOVA results for main factors within each panel are shown.

Chapter 4

Discussion

The objective of this study was to determine if woody plant responses to an abrupt heating event (or heat wave) differ between plants experiencing heating of the shoot only *vs*. shoot and root together (*i.e.*, air heating *vs*. air+soil heating). The limited research on this topic indicated that shoot+root heating is typically more stressful to plants than shoot heating alone, and that there can be indirect effects of heat-stress on the root system (*i.e.*, heat effects on roots in the absence of soil heating). However, prior studies focused on herbaceous species (mostly bentgrass species) (Huang *et al*. 2012), and the single previous study on a woody species (Hao *et al*. 2012) suggested that woody and herbaceous species may differ in their responses. Our results for two species of oak, indicated that plant responses to only air heating differed from air+soil heating, and that there were both direct and indirect effects on roots. Decreases in P_n, leaf mass and area, and fine-root mass during moderate heat-stress (35^{o}C) were actually greater when roots were insulated from heating. Insulating roots resulted in soil temperature differences between insulated and un-insulated soil during heating, with insulated roots having lower *in situ* root respiration, but had little effect on leaf (Figures 3-3 & 3-9) or root water status (data not shown). Hence, the negative effects of root insulation during heating on growth after 8 days of moderate heat-stress were likely correlated with decreases in P_n, but the reasons for the decrease in P_n are unclear (but see below).

4.1 Shoot Effects

Our results indicated that acute heat-stress can affect the physiology of oak seedling shoots. However, some heat-stress effects appeared to be independent of soil temperature, whereas other effects varied depending on the temperature in the root-zone of the plant. For example, direct heating of *Q. alba* and *Q. velutina* caused a significant decrease in G_s (stomatal conductance), but only a mild decrease in Ψ_L (leaf water potential), and these effects were unrelated to soil temperature. Water stress may cause a decrease in P_n due to stomatal closure, but a review on drought tolerance in oaks noted that water related stomatal closure does not occur in these species until Ψ_L reaches -2.30 (*Q. abla*) and -2.45 MPa (*Q. velutina*), which were lower than any Ψ_L values we found in this study (Abrams 1990). Also, the decreases in Ψ_L only occurred with moderate heat-stress (35°C) and could not be correlated to decreases in P_n and G_s. The lack of a relationship between these parameters suggested that changes in the physiological functioning of the shoot were probably not related to water stress and instead were caused by heat alone.

Our data also suggest that high soil temperature can ameliorate aboveground plant responses to heat-stress. For example, heating the soil with the shoot (un-insulated pots) at moderate heat-stress (35°C) in *Q. alba* produced no difference in P_n and C_i from controls. In contrast, heat-stress applied to only the shoot (insulated pots) of *Q. alba* (and *Q. velutina*) decreased P_n and increased C_i, indicating metabolic damage to photosynthesis. Our results were similar to Hao *et al.* (2012) who found that maximum quantum yield of PSII (F_v'/F_m') was only decreased in *Prunus mira* seedlings when the soil was insulated, indicating that damage to PSII was related to low soil temperature during heat-stress to the shoot. In contrast, other studies of herbaceous plants generally found that photosynthesis was impaired more when soil was warmed (Udomprasert et al. 1995; Xu and Huang 2000). At severe temperature heat-stress (40°C), soil temperature had little effect on the photosynthetic response of *Q. alba* to heat-stress, with both P_n and C_i levels dropping consistently for both heated and insulated soil treatments. Indeed, a severe heat-stress can induce different damages to photosynthesis than a moderate stress (Sharkey 2005), and other studies we could find did not define or quantify the heat-stress level experienced by plants (Hao et al. 2012; Udomprasert et al. 1995; Xu and Huang 2000). Our data however, indicated that the photosynthetic response of *Q. alba* and *Q. velutina* to heat-stress is affected by soil temperature, but only at a moderate heat-stress level.

4.2 Root Effects

Root metabolism and biomass were also affected by heat-stress and these effects were only partially dependent on direct heating of the soil. Although a decrease in respiration would have indicated damage to the root system, respiration rates in heated plants showed a slight increase regardless of soil temperature. The consistency of this effect, which is independent of soil temperature, indicates an indirect effect on root function from heat-stress to the shoot, the exact mechanism of which remains unclear. However, the fact that respiration rates were also generally higher at higher incubation temperatures (20°C *vs.* 35°C), shows that increased respiration can be partially explained by the higher temperature of the soil in un-insulated pots, indicating a direct effect of soil warming on root respiration. Although we do not know the cause of the respiration change, a study on sunflower grown at different soil temperatures found increased root respiration with soil temperature was mainly due to higher maintenance respiration (Szaniawski and Kielkiewicz 1982). The only notable difference in respiration rates in the present study was seen in *Q. alba* roots, which were markedly higher at the high temperature incubation (35°C *vs.*20°C). Xu and Haung (2000a) also found high air temperature raised respiration rates of creeping bentgrass in heated soils, but in their study, insulated roots maintained respiration rates similar to controls. Respiration rates also increased from day 4 to day 8 in *Q. velutina*, but the rate of this increase was higher in insulated pots than in un-insulated pots. Data from Xu and Huang (2000a) suggest that the increase in root respiration may be temporary in some species, declining back to control values within 8-13 days. Thus, the duration of the heat-stress may be an important factor influencing temperature effects on root respiration, so future research could examine this effect over a longer duration.

Although root respiration rates increased for all heat treatments, root biomass changed only for *Q. velutina.* There was a marginally significant ($P=0.065$) decrease in fine root biomass with heat-stress (35°C), but this decrease only occurred with low soil temperature (+box). Our results contrast with several studies done on herbaceous species, which showed that root biomass decreased more with combined shoot+root heating than shoot heating alone (Udomprasert et al. 1995; Xu and Huang 2000). A study of *Triticum aestivum* (wheat), however, found a similar decrease in root (and shoot) biomass when shoots were heated while soil temperature was low (35/25°C; shoot/root temperature). This decrease in biomass was only temporary, lasting for the first 14 days of heat-stress, after which growth increased to control values indicating that our biomass response may have only been temporary (Kuroyanagi and Paulsen 1988). Although we only saw small heat effects on biomass in *Quercus* after 8 days, this is likely due to the relatively slow growth rate of woody species. However, lower photosynthate production combined with increased root respiration may have resulted in decreased root and shoot biomass in both heat-stress treatments regardless of soil temperature, if the study had gone on longer.

4.3 Conclusion

The results of this study on oak seedling responses to heat-stress indicated that the level of stress these plants experienced during a heating event was partially contingent on soil temperature. A temperature differential between heated air and insulated soil during heating caused a larger decrease in photosynthesis under moderate heat-stress temperatures, compared to plants experiencing both air and soil heating. The increase in internal CO_2 seen in both *Q. alba* and *Q. velutina* with heat-stress indicated metabolic damage to photosynthesis and occurred only in the insulated pots (no soil heating). The effect we found on photosynthesis was comparable to results of the only other similar study we could find on a woody species (Hao *et al*. 2012), but contradicted the more common studies of herbaceous species (Udomprasert et al. 1995; Xu and Huang 2000). The exact mechanism for this response is unknown, but could indicate that finer nuances of soil temperature regime during heat-stress plays a critical role in the level of stress a plant experiences. A few studies have found that ABA levels increase more in leaves when roots are heated in conjunction with shoots than when roots are heated alone (Hao *et al*. 2012; Talanova *et al*. 2003). Increased ABA levels in leaves are associated with increasing thermotolerance of the shoot, a benefit that has been found in many other plant species although the mechanism for this has not been explained (Kumar *et al*. 2012; Larkindale and Huang 2004). Root respiration rate did tend to increase with heat-stress, indicating a greater carbon cost to the plant, so that an increase in carbon use (respiration) along with a decrease in carbon production (photosynthesis) could reduce the net carbon gain of these seedlings. Future research to understand these synergistic effects during multiple events and how they might impact the long-term carbon dynamics of plants would help determine the cumulative effects of heat waves on trees. The responses observed in this study suggest that heat waves associated with global climate change could have adverse effects on the regeneration, growth, and survival of temperate woody species, and that effects of air heating may differ compared to air+soil heating.

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