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Taking the Heat: Ecophysiological responses of *Heteromeles arbutifolia* to heatwaves

By
Michal Ilana Shuldman

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

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in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Todd E. Dawson, Chair

Professor David Ackerly

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ABSTRACT

Taking the Heat: Ecophysiological responses of *Heteromeles arbutifolia* to heatwaves

by

Michal Ilana Shuldman

Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor Todd Dawson, Chair

In a world of changing climate, changes in the magnitude and timing of fluctuations in environmental conditions are expected to alter plant functioning. Plants are already responding to anthropogenic climate change and in the next hundred years they will face an increase in the severity, duration, and frequency of extreme climatic events, such as heatwaves. To understand the impacts of climatic extremes on plants we need to study the exposure and vulnerability of natural systems in the context of natural climate variability. Few studies have examined plant responses to heatwaves - short-term, intense increases in temperature. Here, the ecophysiology of the California native shrub *Heteromeles arbutifolia* is explored, with the goal of determining the extent of within species variation in ecophysiological responses to heatwaves.

Chapter 1 examines if the ability to tolerate heatwaves varies among distant populations of *H. arbutifolia*. Vulnerability to heatwaves may depend on genetic variation in phenology, growth, reproduction and physiological tolerances, and these factors may vary within a species among different populations. This study showed that two populations of a widespread California native shrub responded differently to an artificial heatwave. In the greenhouse, regardless of treatment, plants from the northern site had higher stomatal conductance and plants from the southern site had higher nighttime respiration. An artificial spring heatwave treatment, when water availability was high but air temperature suddenly increased 5°C, resulted in increased transpiration by plants from both sites but the magnitude of the increase was greater in plants from the northern site. However, our results also show that an artificial spring heatwave did not result in lower photosynthetic rates or higher respiration rates, indicating that high water availability may have allowed plants from both sites to cope with extreme temperatures.

Chapter 2 examines the responses of two growth forms of *H. arbutifolia* to naturally occurring heatwaves in the field. . *H. arbutifolia* is a resprouter, with plants regenerating after a fire from shoots produced by belowground meristems fuelled by non-structural carbohydrate reserves. There is strong evidence that resprouting individuals have higher photosynthetic and transpiration rates than mature non-sprouting individuals. Therefore, vulnerability to heatwaves may depend on plant growth form. This study shows that

when seasonal water availability was lower during a fall heatwave, plants reduced stomatal conductance, but when water availability was higher during a spring heatwave stomatal conductance was unaffected by the heatwave. Resprouts in this study did have higher electron transport rates and higher transpiration rates, yet these differences did not result in mature shrubs and resprouts responding differently to either a spring or fall heatwave. Detecting a strong heatwave response in the field for drought tolerant shrub species such as *H. arbutifolia* may require heatwaves of larger magnitude or heatwaves coupled with severe drought. These extreme heatwaves or combinations of extreme heatwaves and drought are rare and therefore difficult to capture in the field; however, it is just such events that are likely to be the most important to predict plant vulnerabilities as extreme events become more common in the future.

Chapter 3 examines the seasonal patterns of water and carbon use in the two growth forms of *H. arbutifolia.* Most of the research comparing resprouting and mature individuals of a species happens immediately following a disturbance. To assess the relative risks to an obligate resprouting species it is important to understand the long-term performance of both forms of the plant. This study focuses on differences 4-years and 5-years post-disturbance. We found that many aspects of the physiology of two types of had converged but that resprouts had higher photosynthetic rates at the end of the dry season. If differences between mature and resprouting shrubs are more pronounced when leaves are experiencing water limited conditions, this could have important implications during drought years or under projected climate change if the precipitation regime changes.

This research is some of the first to examine the influence of ecotypic variation in response to heatwaves. Overall, these studies show that *H. arbutifolia* is likely to be robust to heatwaves of the magnitude studied. *H. arbutifolia* exhibits within species variation despite growing in a wide range of environments throughout its range. Understanding the magnitude of within species variation between different populations and different forms of the plant (i.e. resprouting and mature individuals) will be important to determine the vulnerability of the species to future climate change.

For Sivan

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INTRODUCTION

"A change in the weather is sufficient to recreate the world and ourselves"

-- Marcel Proust

Plant and animal species are responding to recent, anthropogenically induced climate change (Menzel et al. 2011; Parmesan 2006; Meehl 2004; Parmesan and Yohe 2003; Root et al. 2003); such changes are predicted to continue and have impacts at physiological, demographic, and evolutionary scales (Rosenzweig et al. 2007). The next hundred years will see substantial changes in climate and in the frequency of extreme weather events (Coumou and Rahmstorf 2012; Lavell et al. 2012; Rahmstorf and Coumou 2011; Beckage et al. 2008; Kelly and Goulden 2008; Parry et al. 2007). Significant biodiversity loss may occur if plant species cannot survive under future climate (Peñuelas et al. 2007; Kueppers 2005; Dole et al. 2003;). For example, a recent bioclimatic model linking species current ranges to future warming scenarios estimates that within a century, up to 69% of plant species endemic to the California Floristic Provence will be found in less than 20% of their current range (Loarie et al. 2008). This realization has led to a growing interest in developing adaptation strategies: actions humans can and must take to minimize the impacts of climate change on organisms and ecosystems (Parry et al. 2007). A scientifically based approach for developing adaptation strategies requires accurate forecasting of future climate change and biotic responses on local and regional scales.

We need to understand mechanisms of response in order to scale our predictions to ecosystem function, demography, and ultimately distributions. Traditionally, it is thought that species will shift distributions in response to climate change by tracking their current climate optimum and moving upslope and northward (Hickling et al. 2006; Parmesan 2006; Root et al. 2003). This general pattern may not hold well for many species in California, where due to a sharp coastal to inland transition, moving coastward may result in a cooler climate than moving northward (Loarie et al. 2008). In particular, variability in temperatures between seasons increases dramatically from coastal to inland sites and seasonal water availability is much greater in the north.

My dissertation research set out to examine within species variation in the ecophysiology of a widespread shrub found growing throughout the strong environmental gradients of the California Floristic Provence. Understanding the role of within species variation is an important because if populations are well adapted to local environments, then moderate to strong climate change is likely to cause significant declines in productivity and fitness, as genotypes are stuck in suboptimal conditions. This is especially true for woody plants that take years to reproduce and disperse. In addition, within species variation is virtually ignored in one of the major techniques used in forecasting species distribution (Elith and Leathwick 2009; Loarie et al. 2008; Botkin et al. 2007; Kueppers 2005). Species distribution models (SDM) aim to forecast where a species will persist in the future and if that location falls within the climate envelope described by the species' current range. While SDM can provide a first approximation of where a species may live in the future, they overlook important ecological and evolutionary factors that may be important for persistence (Sinclair et al. 2010; Wiens and Bachelet 2010). Despite these limitations, SDM are one of the few practical approaches now available for forecasting future species distributions. Observations and experiments are needed to help improve our knowledge of which limitations may be the most critical for survival and persistence (Elith and Leathwick 2009).

In the future, the number of extreme weather events, such as heatwaves, will increase (Lavell et al. 2012), yet we know very little about how plants will respond to an increase in frequency, duration or severity of heatwaves. In California the duration of heatwave season is expected to increase by up to thirteen weeks, yet it remains unclear whether it will start earlier, end later, or both (Hayhoe 2004). The seasonal timing of heatwaves will likely have different effects on native plants. In the Mediterranean-type climate of California, sudden heat in the spring, when soil water availability is high, should affect plants differently than extreme heat at the end of summer, when plants have acclimated to high temperatures but soil water availability is low. Moreover, the responses of native species might be different across their ranges if they are adapted to the local climate. The responses of native species may also vary during different life stages. For example, many California shrubs resprout from belowground stems after a crown-destroying fire (Keeley et al. 2012) and these resprouts are know to have increased photosynthetic rates compared to mature shrubs (Keeley et al. 2012).

Numerous studies have reported on the effects of increased average temperatures on plants, but very few have investigated the effects of differences in climate variability. The ability to tolerate extreme events may vary among populations depending on genetic variation in phenology, growth, reproduction and physiological tolerances. This research is some of the first to examine the influence of within species variation in response to climate change, and to heatwaves specifically. In Chapter 1, I examine the responses of seedlings from two populations of *Heteromeles arbutifolia* to an artificial spring heatwave. I use the common garden approach (Clausen et al. 1940) of collecting seeds from multiple locations and growing the seedlings together in a common environment. The common garden approach assumes that when plants are grown together any differences found between sites are due to differences in genetic makeup (assuming no maternal effects). I expanded on this chapter with an additional experiment that determines ecophysiological responses to an artificial spring heatwave in coastal and inland populations *H. arbutifolia* from northern and southern California; however; those results are not presented here. In Chapter 2, I examine the response of resprouts and mature shrubs growing in the field to naturally occurring spring and fall heatwaves. In Chapter 3, I examine the ecophysiology of the same resprouts and mature shrubs to gradual seasonal changes in temperature and water availability.

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CHAPTER 1

Ecotypic differentiation in response to an artificial heatwave in a California native shrub

Abstract

The number of heatwaves, short but intense increases in temperatures, is expected to increase in California in the future. To determine the magnitude of likely threats to biodiversity from an increase in the severity or duration of the heatwave season, and to provide a strong scientific basis for conservation and management strategies, we must be better able to forecast responses to climate change. Numerous studies have reported on the effects of increased average temperatures on plants, but very few have investigated the effects of heatwaves. The ability to tolerate extreme events may vary among populations depending on genetic variation in phenology, growth, reproduction and physiological tolerances. This study showed that two populations of a widespread California native shrub responded differently to an artificial heatwave. In the greenhouse, regardless of treatment, plants from the northern site had higher stomatal conductance and plants from the southern site had higher nighttime respiration. An artificial spring heatwave treatment, when water availability was high but air temperature suddenly increased 5°C, resulted in increased transpiration by plants from both sites but the magnitude of the increase was greater in plants from the northern site. The differences in transpiration rates between plants from the two sites when they were grown in a common environment indicate that there is some ecotypic variation in physiology in this widespread species. However, our results also show that an artificial spring heatwave did not result in lower photosynthetic rates or higher respiration rates, indicating that high water availability may have allowed plants from both sites to cope with temporary heatwaves. Local adaptation may cause populations to decline or persist in ways that cannot be captured with forecasting based on models that do no incorporate within species variation.

Introduction

For the biodiverse California Floristic Province, heatwaves are one of several important threatening extreme events that merit study. Heatwaves are vaguely defined as unseasonably high temperatures and which may affect the performance and dynamics of terrestrial plants (Borken and Matzner 2009; De Boeck et al. 2010; Jentsch et al. 2011; Smith 2011). While there has been no single definition of a heatwave (Robinson 2001; Souch and Grimmond 2006; Gershunov 2009), we define a heatwave as an episode in which the daily maximum temperature exceeds the long-term average maximum temperature by 5°C for at least 4 consecutive days (Frich 2002). High temperatures can affect the carbon balance of a plant by damaging the photosynthetic machinery, destabilizing membranes, or increasing respiration rates (Lambers et al. 2008). At the same time, high temperatures can affect the plant water balance if the plant closes its stomata to prevent water loss (a typical response when heat and drought are experienced in concert) or if transpiration increases, which it may in part due to the direct effects of high temperatures on evaporation, and which would facilitate evaporative cooling of leaf surfaces (Lambers et al. 2008). When soil water is abundant after the winter rains, plants may be able to increase transpiration and latent heat loss to counteract some of the effects of increased air temperatures. In contrast, when soil water is limited after summer drought, plants may be unable to transpire if drought induces water stress. Plants experiencing water stress are more likely to close their stomata while also up- or down-regulating biochemical and enzymatic processes (Rennenberg et al. 2006) or they may simply tolerate negative water potentials. Thus, differences in water availability between spring and autumn heatwaves may lead to different physiological responses.

Models cited by the IPCC (Lavell et al. 2012) predict that climatic extremes such as heatwaves will increase in frequency and will occur over longer periods each year (Meehl 2004; Barriopedro et al. 2011; Orlowsky and Seneviratne 2011; Rahmstorf and Coumou 2011; Mastrandrea et al. 2011). The seasonal timing, duration, and maximum temperatures achieved during heatwaves should all have major impacts on plant function (De Boeck et al. 2010). In California (CA), the heatwave season is projected to increase by 5-13 weeks (Hayhoe 2004), yet it remains unclear whether this type of season will start earlier in the spring, end later in the fall, or both. Moreover, these two possibilities would likely have different effects on plants. Because there is often a strong link between drought and heatwaves, understanding the timing of heatwaves could be critically important. In California, spring temperatures are cool and soil water availability is high while in fall temperatures are high and soil water availability is low. If the heatwave season starts earlier in the spring then plants will not have acclimated to high temperatures and an early season spring heatwave could have greater physiological impacts on the photosynthetic machinery compared to a late season fall heatwave. Alternatively, if water availability is more important than temperature acclimation then fall heatwaves may have greater impacts on plant performance. In the study we examine the effects of a spring heatwave, i.e. a sudden heatwave when days are still short and water availability is high. Studying a spring heatwave allowed us to determine the potential plasticity of plants that have not acclimated to the high temperatures and low water availability that would be common during the summer or fall.

The objective of the current investigation was to determine whether geographically distinct populations of a widespread plant species differ in their physiological tolerances to climate change. In particular, we wanted to distinguish between the effects of phenotypic plasticity and local adaptation on the physiological responses to extreme heat events (heatwaves) of the widespread, California shrub *Heteromeles arbutifolia* (Rosaceae) that is common throughout the California Floristic Province.

The lack of information on within species variation in physiological parameters is especially problematic for predicting future outcomes of widely distributed species that experience a broad range of environmental conditions. Populations of these species can cope with varying environmental conditions through either phenotypic plasticity or ecotypic differentiation and local adaptation (Gimeno et al. 2009). Phenotypic plasticity influences the range of phenotypes a single genotype can express as a function of its environment (Nicotra et al. 2010). Local adaptation occurs when different genotypes have different phenotypes and the local adaptation could be driven by differences in the environment between sites. Whether a population exhibits plasticity or adaptation will affect how the population responds to climate change. Species that exhibit high plasticity in the face of climate change are more likely to persist in their current locations than species that exhibit high local adaptation (Parmesan 2006; Valladares et al. 2007). In order to accurately forecast plant responses to climate change we need to understand much more about the roles that plasticity versus local adaptation play in plant responses to expected climate change (Climent et al. 2008).

The mechanisms involved in climatic tolerance are dependent on gene expression, providing an opportunity for natural selection to lead to local adaptation to different selective pressures found in contrasting climatic conditions (Savolainen et al. 2007; Nicotra et al. 2007; Gimeno et al. 2009). While some recent models incorporate intraspecific variation in physiological responses to environmental changes (Kearney and Porter 2009; Orlowsky and Seneviratne 2011l; Stratonovitch et al. 2012), we currently do not understand the relative importance of phenotypic plasticity compared to local adaptation for this generation of models to be widely applied and to accomplish what current SDMs cannot. One area of concern is how broadly distributed species, which may be locally adapted, respond to extreme weather events. Populations already struggling at the edge of the suitable climate are likely to be the most vulnerable. At the same time, local adaptation shows the species is capable of evolutionary responses to local conditions, so adaptive evolution could be an important response to climate change. While many woody plants will not have a fast enough generation time to see evolutionary responses on the century scale, over the course of multiple centuries evolutionary responses are possible in in shrubs and fast-growing trees.

Our study had two parts. In the first part we compare the physiology of adult plants from two distant sites in the field during the spring and the fall. We expected that the plants from the southern site would exhibit more conservative water use strategies because the site receives an order of magnitude less rain than the northern site and is warmer during the spring. The adult plants from these two sites were used as seed sources for the second part of the study.

In the second part of our study we exposed greenhouse-grown seedlings to an artificial spring heatwave, when soil water availability is high but atmospheric demand for water can increase abruptly due to increasing temperatures during a heatwave. Our artificial heatwave aimed to increase maximum temperatures by at least 5°C above the average maximum temperature for at least four days. By growing seedlings from two different sites together in the same environment for three years and then exposing them to an artificial heatwave treatment we were able to separate the effects of plasticity and adaptation. If plants from both sites responded similarly to the heatwave treatment, for example both decreasing stomatal conductance, this indicates no ecotypic variation, so any differences observed in the field would be due to plasticity. If plants from both sites response differently to the heatwave, for example the northern site always has higher conductance than the southern site, this indicates the two sites are locally adapted and differ genetically. If the response to the heatwave treatment is dependent on which site the plants are from, for example plants from both sites decrease stomatal conductance but the northern site has a more dramatic response, this indicates that both plasticity and adaptation play a role (i.e. gene X environment interaction).

We expected that when exposed to artificial spring heatwaves, seedlings from southern CA would respond differently than plants from northern CA because of local adaptation to the unique climates at the latitudinal extremes of the species range. However, with only one population from each latitude, any ecotypic differences could reflect latitude (and therefore temperature) or something else that varies between sites, such as soil conditions. This study will determine if ecotypic variation is present in plants from two distant sites, even if it cannot explain the exact mechanism of the difference. Further research on multiple populations from each latitude is needed to provide definitive evidence that ecotypic differences are due to differences in climate.

Based on this, we predicted that compared to pre-heatwave conditions, seedlings from both populations would show increased transpiration, increased respiration, increased stress to the photosystem II (estimated from fluorescence) and lower photosynthetic rates when exposed to an artificial heatwave. In addition, we predicted that while seedlings from the southern site would increase their transpiration rates during the heatwave, they would have a more conservative water-use strategy and the magnitude of the increase would be smaller than the northern site. In other words, we expected that physiological response to an artificial heatwave would depend on the environment the plants came from and that the response would result in an increase in water loss and a decrease in carbon fixation.

Materials and Methods

Study System

H. arbutifolia is a wide-spread shrub found throughout the California Floristic Provence and inhabits coastal shrublands, inland chaparral, and montane forest understories. We surveyed two field populations, approximately 1000 km apart in Mendocino and San Diego County, to determine if there were differences in carbon and water use in adult plants at the two sites. Then we collected seed from the two locations and grew the plants together in a common environment. The “common garden” approach is one way to determine if population differences are due to plasticity or adaptation. If plants grown together have the same phenotype and physiology then any differences found in the field are due to plasticity and not to adaptation. Finally, we exposed three-year-old seedlings to an artificial spring heatwave in the greenhouse.

Species Description

H. arbutifolia has intermediate rooting depth (Davis and Mooney 1986; Ackerly 2004) and our previous research has shown that it experiences substantial water deficit in midsummer suggesting it could be an indicator of site water availability since it does not tap into deep water pools. Unlike many chaparral shrubs it has large leaves. It is insect pollinated and the fruits ripen and turn bright red in late fall. The fruits are primarily bird dispersed but are also consumed by bears and coyotes. Plants resprout vigorously after fire, and seedlings only establish in the understory during fire-free intervals (McMurray 1990).

Site Descriptions

The northern site was located at the Heath and Marjorie Angelo Coast Range Reserve (ACRR) in Mendocino County (39 N 43'50, 123 W 38'40). Despite being located only 24 km east of the Pacific Ocean the ACRR lies east of Elkhorn Ridge, a high region of the coast range, and is shielded from maritime fog. Consequently, it has greater temperature extremes and drier summers than might be expected from a site located in close proximity to the ocean. The ACRR has steep, dissected terrain (378-1290 m) made up of redwood groves, upland Douglas fir and mixed conifer-deciduous forests, meadows on upland river terraces, and chaparral at higher elevations, particularly along ridgelines. *H. arbutifolia* is an uncommon understory shrub found primarily in upland Douglas fir and mixed conifer-deciduous forests.

Our field site was located on a ridge top approximately 4.8 km past the entrance to the Conger Trail. Most of the plants we selected were growing in the understory of the mature surrounding forest. The soil is from the Holohan-Hollowtree-Casabone complex (http://casoilresource.lawr.ucdavis.edu/drupal/node/902), which is a well-drained Alfisol (suborder Xeralf) weathered from sandstone.

The southern site was located at the Elliott Chaparral Reserve, in San Diego County (32 N 53'29, 117 W 05'41). The site is located approximately 9.6 km from the Pacific Ocean and experiences a strong maritime influence. Elliot Chaparral Reserve includes a mixture of both coastal and desert habitats with a wide variety of coastal chaparral and coastal sage scrub (61-305m). The reserve is primarily made up of rolling topography of broad valleys and arroyos as well as the narrow, steep-sided ridge of Kearny Mesa. *H. arbutifolia* is an uncommon shrub found primarily in valley bottoms.

Our field site was located in a valley bottom near an arroyo that floods during the winter rains. The plants were growing in an open shrubland and therefore received much more light than the plants growing in the understory at the northern site. The soil at the site is a well-drained Alfisol (suborder Xeralf) from the Redding-Oliventain-Huerhuero complex. The soils are alluvium derived from mixed sources and are thin, pebbly, and leached. The chaparral plants, particularly chamise, are more stunted and there are larger spaces between plants than in most other Southern California locations. The site burned in the 2003 Cedar Fire. Consequently, the plants at this site were all resprouting individuals. The shrubs were independent, growing far enough apart that clonal growth is unlikely. One of the plants died and was removed from the analysis in 2010.

Average air temperature and maximum air temperature were calculated from the Western Regional Climate Center using the closest station to the field site for 2005-2012 (Figure 1); Camp Elliot station (32 N 51'33, 117W 06'20) for the southern site and Laytonville station (39N 42' 08, 123W 29' 06) for the northern site (Figure 1). The two sites have a similar maximum air temperature in the summer, but it is much warmer on average at the southern site in the spring and winter.

Field Sampling

We randomly selected 10 adult shrubs at each site and studied the same plants from the end of the dry season (Summer) in 2008 to the end of the dry season in 2010. In 2009 and 2010 we also sampled the plants at the end of the wet season (Spring). During the sampling periods we collected predawn and midday leaf water potential using a Scholander-style pressure chamber (Plant Moisture Stress, Albany, OR). Leaves were excised, immediately wrapped in plastic wrap, put in a small ziptop bag, and placed in a dark cooler until they were measured (Jacobsen et al. 2008). Leaves remained in the cooler for less than 90 minutes. Data gathered at the University of California, Berkeley campus using the same method indicated that there was no significant difference in water potential for leaves on the same branch if they were measured immediately after sampling or after sitting wrapped and in the dark cooler for 90 minutes (data not shown). We measured steady state stomatal conductance using SC-1 steady state porometer (Decagon Devices, Pullman, WA) on two adjacent leaves, on one of which we also measured leaf water potential. We measured the photosynthetically active radiation (PAR) at the leaf surface using a quantum sensor held parallel to the ground (LI-250A, Li-COR Biosciences, Lincoln, NE). We sampled leaves at predawn to test for the potential quantum yield of photosystem II by measuring fluorescence with a Walz Mini-PAM (Heinz Walz GmbH, Germany). At predawn the light pulse on the fluorometer was set to low. We measured the relative humidity at the leaf surface using a handheld sensor (Humidity and Temperature Indicator HMI 31, Vaisala, Finland). To determine leaf temperature we used a standard procedure to make a type-T fine wire thermocouple (constantan-copper) and connected it to a voltmeter that outputs temperature. The measurements were taken by touching the thermocouple to the bottom of the leaf and sampling the air near the leaf. Not all measurements were taken at each sampling period; see Table 1 for a matrix of sampling periods and measurements. We logged temperature and humidity in the plant canopies continuously (EL-USB-2, Lascar, United Kingdom) in 2009-2010.

Seed Collection and Growth

We collected fruit from the southern site December 14, 2008 and from the northern site December 28, 2008. The fruit was clipped from the plant, placed in a ziptop bag, and kept in a cooler until it could be refrigerated. At the University of California, Berkeley the seed was extracted from the fruit using a blender with the blades covered to break up the fruit. Then the seeds were picked by hand from the slurry and placed in a container of clean water. The seeds were left in the water for 5-7 days. Then they were washed with a dilute bleach solution (1:10), rinsed with distilled water, and immediately planted in germination flats (January 6-16, 2009). The soil used was a custom blend of 6 parts loamy sand (American Stone and Soil, Richmond, CA), 4 parts peat moss (Sunshine Peat Moss), superphosphate (Green All, E.B. Stone, Suisun, CA), and calcium carbonate (Fisher Scientific). Plants were grown in a greenhouse at the University of California, Berkeley.

Growth and Physiology

We measured germination and leaf number on ~400 seedlings starting every other day and decreasing the frequency over time as the plants grew (data not shown). We transferred the plants from the germination flats to D40 Deepot containers (Stuewe and Sons, Tangent, OR) in February and March 2009. Starting in September 2009 we selected a subset of plants to be repotted into 1-gallon pots.

Greenhouse Experiment

We randomly assigned 3 plants from each site into 21 groups (126 plants total). Each Heat, No Heat, or Ambient group was randomly assigned to a position in the greenhouse (i.e. a completely randomized design) and placed on standard greenhouse benches. The overhead high-intensity discharge lamps were turned on in the greenhouse. One set of 7 groups was left in the greenhouse (Ambient) and the other 14 groups were each placed in their own chamber (i.e. each chamber is a replicate and n=7). The 14 chambers were made from a frame of PVC pipe covered in 0.001" thick Tefzel® Optically Clear Film (CS Hyde, Lake Villa, IL) on all sides except the bottom. Each frame was 153 cm tall, 86 cm wide and 67cm deep. Each chamber had a Bayco 10.5" Brooder Clamp Light (Walmart) attached at the top. Half of the chambers (Heat) had a 250W Exo Terra Ceramic Heater (Hagen Corp, Mansfield, MA) installed in the light and the other half of the chambers (No Heat) had the light without a heater. Each chamber had two computer fans (MASSCOOL 80 mm Case Fan, Fanner Tech USA Corp., City of Industry, CA) positioned near the top of the chamber, slightly below the clamp light. from 07:00-15:00 if the incoming radiation dropped below 300 Wm^{-2}, otherwise the lights remained off.

Each chamber had a data logger placed under a radiation shield continuously logging temperature and relative humidity (EL-USB-2, Lascar, United Kingdom). The average temperature was at least 5°C warmer, the relative humidity was lower and the vapor pressure deficit was greater in the Heat treatments during the heatwave periods. During the recovery period between heatwaves there were no differences in temperature, relative humidity or vapor pressure deficit among the different treatments (Supplementary Figure 2).

We sampled plants during four periods: Pre-treatment (8 days), Heatwave I (6 days), Recovery (4 days), and Heatwave II (5 days). During each period plants were sampled at the beginning (day 1-2) and end of the period (day 5-6). At each of these time points we measured midday stomatal conductance and transpiration with a LiCOR 1600 (LiCOR Biosciences, Lincoln, NE) on one plant from each site in each group (n=7; 7 chambers of 3 treatments is 42 plants total). We measured predawn and midday fluorescence on three plants from each site in each group (n=7, 126 plants). On the second day of the Pre-treatment, Heatwave I and Heatwave II period we measured maximum midday photosynthesis and nighttime respiration with a LiCOR 6400 (LiCOR Biosciences, Lincoln, NE) on one plant from each site in a subset of the groups (n=4, 24 plants). Maximum photosynthesis was measured by ramping up the light from 500-1500umol in 500umol increments. The LiCOR 6400 was placed in a chamber and measurements were not taken until the block, leaf, and air temperatures were stable. We started respiration measurements 1 hour after the lights in the greenhouse turned off. We measured the volumetric water content of the soil during each of the 4 periods (Hydrosense, Campbell Scientific, Logan , Utah) and adjusted the water given to all the plants so that water content was greater than 35%.

Statistics and Calculations

For the data from the field, ANOVA was performed in R and any interaction terms were investigated further using post-hoc pairwise t-tests with a Bonferroni correction. Due to chance the plants in the Ambient group of the greenhouse experiment had significantly higher rates of photosynthesis and transpiration during the pretreatment and treatment phases of the experiment. We opted to remove these plants from the analysis and calculate the difference between individual plants in the heat wave treatment groups during the treatment period and pretreatment period. If measurements were taken on more than one day of pretreatment then we used the average of those days. We then performed our statistical analyses on the differences in JMP using standard least squares regression and the REML method (SAS Institute). The model used Site and Treatment as fixed factors and Chamber as a random factor.

Results

Field Results

Predawn leaf water potential (Figure 2A) was not significantly different between southern and northern sites in spring (P=0.059) but the southern site had less negative water potential in the summer (i.e. plants were more well-hydrated, P=0.005). There was no significant difference between the different years of the study. Midday leaf water potential (Figure 2B) was more negative for both sites in summer than the spring in all years. Post-hoc pairwise t-tests showed that the southern site had more negative midday water potential in spring (P<0.001) but that there was no significant difference between the two sites in summer (P=0.81). In both spring and summer 2010 plants from the southern site had higher light at the leaf surface (Supplemental Figure 1A) and a lower leaf-to-air vapor pressure gradient (VPG) than the northern site. But the VPG was higher in the summer period compared to the spring at both sites (Supplemental Figure 1B). In the spring, when the sites have similar soil water availability, there was no difference in stomatal conductance (P=0.64). However, in both years during the summer, when the southern plants have more soil available water, the plants had higher midday stomatal conductance (P<0.005, Figure 3). Also, while conductance at both sites was lower in summer compared to spring in 2009, this was not true in 2010. In 2010 the northern site had higher predawn Fv/Fm (Figure 4) than the southern site. In addition, at both sites Fv/Fm was greater in summer than in spring.

Experimental Heatwave Results

In the greenhouse we found that there were greater differences between the sites in water use (i.e. transpiration and conductance) than in carbon fixation (i.e. photosynthesis and respiration), and this effect was exacerbated by the heatwave treatment. There were no differences in daytime transpiration between sites or treatments during the first heatwave (Figure 5). During the second heatwave the northern site had higher daytime transpiration than the southern site (*P*=0.03); Figure 5). Plants that experienced the artificial heatwave had higher daytime transpiration compared to plants that received no heat treatment (*P*=0.0037). All plants exposed to heatwaves had higher nighttime transpiration during both the first and second treatments (Figure 6). There were no differences between sites or treatments for conductance during the first heatwave but during the second heatwave conductance was higher in plants from the northern site in both treatments (*P*=0.04, Figure 5).

Plants from the two sites did not differ significantly in photosynthetic rates before or during the experimental period and the heatwave treatment had no significant effect on photosynthesis overall (Figure 7). The southern site had higher dark respiration rates than the northern site during the second heatwave (*P*=0.0274), but there was no effect of the heatwave treatment on dark respiration (Figure 8). There were also no differences between the two sites in the photochemical efficiency of photosystem II in darkness (i.e. predawn Fv/Fm) as well as no overall effect of the heatwave treatment on photochemistry (Figure 9).

Discussion

The results reported here show that differences between plants from two distant populations persist in subsequent generations grown in a common environment. In the field there was no difference in stomatal conductance during the spring, when the sites have similar soil water availability; however, in both years during the summer, when the southern plants have more available water (see below), the southern plants had higher midday stomatal conductance. In the greenhouse, regardless of treatment, plants from the northern site had higher conductance and plants from the southern site had higher nighttime respiration. An artificial spring heatwave treatment resulted in increased transpiration in plants from both sites but the magnitude of the increase was greater in plants from the northern site. The differences in transpiration rates between the two sites when they were grown in a common environment confirm our hypothesis that the response to an artificial heatwave was site dependent, indicating that there is some ecotypic variation in physiology in this widespread species. However, our results also show that an artificial spring heatwave did not result in lower photosynthetic rates or higher respiration rates, indicating that the carbon-balance of the plants from both sites was unaffected by the heatwave, possibly because water availability was high.

Environmental conditions and water-use in field populations

Since we were interested in examining the potential for ecotypic variation in a widespread species we selected plants from two populations growing in very different sites. The southern site had higher light, higher leaf-to-air vapor pressure differences, lower precipitation, and more moderate winter and spring temperatures than the northern site (Figure 1). These are all components of the environment to which local populations could be adapted. Adult plants at the southern field site did not have lower stomatal conductance than northern plants (Figure 3) despite experiencing less rain than the northern plants (Figure 1). In the spring, when soil water availability was similar between the two field sites, the southern plants had similar conductance to the northern plants. In the summer, the southern site actually had higher soil water availability than the northern site, despite significantly less rainfall, and higher conductance than the northern site (Figure 3). This suggests that the southern plants have access to a more stable water source than the annual rainfall at the site would have predicted. The plants at this site were growing near a wash that floods during the winter, which could result in the soil storing water (Atchley et al. 1999). The northern plants were growing on a ridge at site that has been shown to have rapid drainage of water along fractures to groundwater sources (Salve et al. 2012). We collected seed and grew plants from these two sites together in the greenhouse. By using the "common garden" approach (Clausen et al. 1940), we determined that there were genetic differences that underlie their different physiology and, furthermore, that these differences influence their responses to an artificial spring heatwave.

Water-use and carbon responses to artificial heatwaves

After exposing three-year-old seedlings grown in the greenhouse to two artificial spring heatwaves we found that differences in transpiration and stomatal conductance between the heatwave and pre-treatment plants were significantly higher for plants from the northern site during the second heatwave. This confirmed our hypothesis that the seedlings from the southern site, which received less rain, would conserve water. In light of the fact that the southern site had higher soil moisture, despite much less rain, our results suggest that plants from the southern site were adapted to local climate, but not to the particular microsite. More research would be needed comparing plants from drier microsites in the region to confirm this. High transpiration rates can increase evaporative cooling and keep leaves from overheating (Gates 1968). The lack of evaporative cooling can exacerbate leaf damage at air temperatures far below lethal values because the leaf surface can be several degrees warmer than air temperature (García-Plazaola et al. 2008). Seedlings at the southern site may be more susceptible to leaf damage from heat since those seedlings did not increase daytime transpiration during the heatwaves. However, we did not see any evidence that our experimental heatwave caused damage to photosystem II, a reduction in photosynthesis, or an increase in nighttime respiration (Figures 7, 8, 9). More research is needed to determine if the carbon gain in the southern plants would decrease from more severe heatwaves or from fall heatwaves that occur when plants have experienced months of soil drought.

Overall, the heatwave treatment affected plant water use more than plant carbon fixation. We saw increased daytime transpiration in the northern population during the second heatwave and increased nighttime transpiration in both populations. Increased rates of transpiration can have ecosystem level effects. For example, during the 2003 heatwave in Europe surface heating was twice as high over a forest compared to a grassland because the grasses had higher transpiration rates and thus higher latent heat loss (Teuling et al. 2010). However, prolonged up-regulation of transpiration by grasses decreased soil water stores and eventually led to higher surface heating than the forests (Teuling et al. 2010). Increased transpiration during a short-term heatwave can lead to long-term consequences for soil water availability. If in the future spring heatwaves increase they may reduce soil water stores earlier in the season (Cleland et al. 2007; Cleland et al. 2006; Parmesan and Yohe 2003; Root et al. 2003). Reduced soil water stores coupled with higher average temperatures during the summer dry season and/or an increase in fall heatwaves at the end of the dry season could lead to negative impacts for these plants. This is especially true for the northern populations since our field data indicate these plants experienced larger soil water deficit at the end of the dry season. Further research is needed to see how the different populations may respond to combined heatwave and soil drought treatments.

The importance of extreme events

Extreme events such as heatwaves are predicted to increase in many ecosystems and short-term extreme heating events have been shown to have long lasting effects on ecosystems (Lavell et al. 2012; Barriopedro et al. 2011; Orlowsky and Seneviratne 2011; Rahmstorf and Coumou 2011; Meehl 2004). For example, the heatwave and corresponding drought in 2003 in Europe led to an increase in forest fires in Portugal resulting in 5% of the land area being burned (Garcia-Herrera et al. 2010). In addition, there was a 30% reduction in gross primary productivity over Europe resulting in the area becoming a net carbon source instead of a carbon sink (Ciais et al. 2005). The reduction in productivity also included declines in crop yields such as a 36% reduction in maize production in the Po valley of Italy where extremely high temperatures prevailed (Ciais et al. 2005). Despite the impacts that heatwaves can have on natural plant communities and agriculture, we know very little about how species will respond to an increase in duration of the heatwave season (i.e. starting earlier or ending later), in frequency of heatwaves (i.e. fewer recovery days between repeated heatwaves), or in severity of heatwaves. Moreover, the information that is available on plant responses to heatwaves rarely includes direct measurements of plant physiological responses (Rennenberg et al. 2006). Our study is one of the first to examine the response of photosynthesis, fluorescence, respiration, and transpiration to heatwaves.

The importance of plasticity vs. local adaptation.

Actually determining if local adaptation is present is very involved. Plants from different locations need to be grown together in a common environment (i.e. common garden study) to eliminate environmental effects on growth and physiology. Even better would be to grow plants from multiple sites together in different environments, for example in a reciprocal transplant study (Clausen et al. 1940) or provenance trial (di Matteo et al. 2012; Rehfeldt et al. 2002; Rehfeldt et al. 2001; Rehfeldt et al. 1999). If when grown together plants from different sites have different phenotypes, this indicates that local adaptation is important for the species. However, if different populations have the same phenotype (i.e. northern and southern populations look the same), then there are two likely outcomes: (1) there are no genetic differences between plants from the two populations, or (2) there are genetic differences between the populations but not in traits measured in the study. We found that photosynthesis and predawn Fv/Fm, were identical between the two sites when grown in a common environment. This indicated that there was no local adaptation for these traits, as the phenotypes were the same; however it also indicated that the plants were able to compensate for the higher temperature of the heatwave treatment, or that the heatwave treatment was not extreme enough, because there was no effect of the heatwave treatment on photosynthesis. These results do not confirm our hypotheses that (1) seedlings from different populations with contrasting climate would show different photosynthesis responses, nor (2) the heatwave treatment would decrease photosynthetic rates. If plants do not exhibit adaptation to local climates it may reflect opposing selection pressures to complex environmental conditions (Gimeno et al. 2009). Despite finding no difference between sites in carbon gain, we did find that the plants from the southern site consistently had higher respiration, regardless of treatment. This suggests that there was local differentiation in respiration and that the net carbon gain was lower in the southern plants. We also found evidence for local differentiation in water-use, with the plants from the northern site increasing transpiration rates during the second heatwave.

Other studies have found evidence for local differentiation in plant physiological traits. A study of three populations of *Quercus ilex* found that leaf ecophysiological traits (e.g. photosynthetic rates) as well as morphologic traits (e.g. leaf thickness) could be linked to the climate of the seed sources (Gratani et al. 2003). At the same time, other studies have found no local adaptation. In a recent study that used the same *Quercus* species from different source locations, researchers found no differences between 6 sites in assimilation, instantaneous water use efficiency or thermal tolerance in 5-month-old *Quercus ilex* seedlings (Gimeno et al. 2009). In addition, three temperate deciduous tree species grown in a common garden showed little evidence that respiration rates depended on adaptation to the climate of origin (Lee et al. 2005). Both of these studies suggest that there was no local adaptation. More research is needed to determine the prevalence of local adaptation among more diverse species.

Despite the number of studies that have looked at local adaptation in physiological traits, few combine studies of adaptation in conjunction with climate change variables in woody plants. One exception is a recent study that shows the European heatwave of 2003 shortened the growing season in low elevation forests due to increased summertime evapotranspiration and increased the growing season in high elevation forests. Trees from areas with a reduced growing season had reduced stem growth but similar leaf phenology, indicating that leaf phenology was related to the elevation (Jolly et al. 2005).

Conclusions

Heatwaves are expected to increase dramatically in California in the future and pose a grave threat to biodiversity and to human welfare. To determine the magnitude of likely threats, and to provide a strong scientific basis for conservation and management strategies, we must be better able to forecast responses to climate change. Numerous studies have reported on the effects of increased average temperatures on plants, but very few have investigated the effects of differences in climate variability. The ability to tolerate extreme events may vary among populations depending on genetic variation in phenology, growth, reproduction and physiological tolerances. This study showed that a widespread plant exhibits local adaptation in water use. More research is needed to determine if these responses hold during a summer heatwave, when plants experience drought in concert with a heatwave. In addition, more research is needed that highlights the role of phenotypic plasticity compared to local adaptation. Understanding the relative role of phenotypic plasticity and local adaptation in driving species dynamics under climate change can inform the next generation of SDM. Improved models will be critical for providing a scientific basis for developing adaptation strategies that require accurate forecasting of future climate and species locations on local and regional scales.

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Figures

Figure 1. Average monthly air temperature and precipitation from 2005-2012 for the southern (A) and northern (B) sites. Data from Western Regional Climate Center stations Elliot Camp and Laytonville. The southern site (mean annual rainfall = 262 mm) has less rainfall than the northern site (mean annual rainfall = 1447 mm). The northern site experiences a much wider range of temperatures than the southern site.

Figure 2. Predawn (A) and midday (B) water potential from adult plants in the field at the southern (open circles) and northern sites (filled circles). The southern sites has less negative predawn water potential in the summer of 2008 and 2009, but there was no difference between the sites in the summer of 2010. There was no significant difference between the two sites in the spring of 2009 or 2010. The effect of site on midday water potential depended on the season.

Figure 3. Midday stomatal conductance from adult plants in the field at the southern (open circles) and northern (filled circles) sites. The southern site had higher midday stomatal conductance in the summer of both years but there was no difference between sites in the spring.

Figure 4. The maximum quantum yield of PSII in a dark adapted leaf was measured at predawn on adult plants in the field from the southern (open circles) and northern (filled circles) sites. The southern site had values below 0.8, which indicates they are experiencing some stress in the spring and the summer.

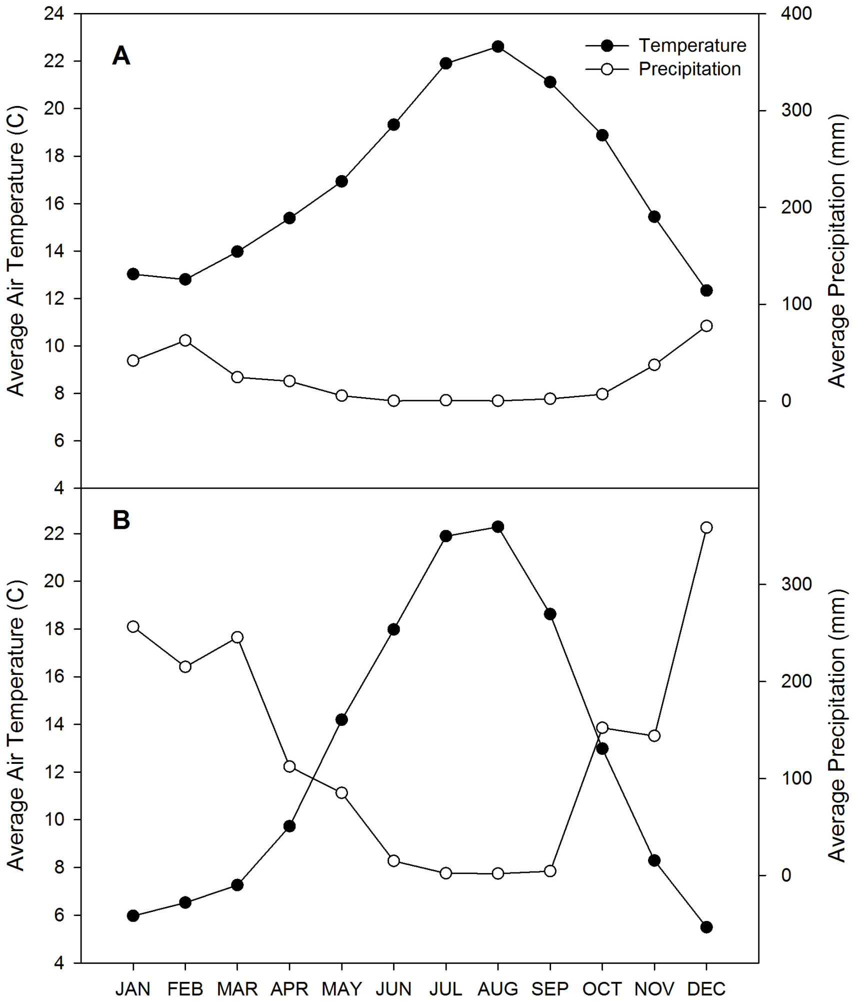
Figure 5. Midday transpiration and stomatal conductance measured with a LiCOR-1600. Transpiration was significantly higher for the northern plants during the second heatwave (0.036). Conductance was significantly higher in the northern plants during the second heatwave but there was no treatment effect. There were no significant site or treatment effects for either conductance or transpiration during the first heatwave.

Figure 6. Nighttime transpiration measured with a LiCOR- 6400. During the first (P=0.0006) and second (P=0.047) heatwaves nighttime transpiration was higher in the heatwave treatment plants, but there were no significant difference between the two sites.

Figure 7. There were no significant differences between sites or treatments in midday photosynthetic rates.

Figure 8. Nighttime respiration was measured one hour after sunset. There were no treatment effects but respiration was higher in the southern plants during the second heatwave (P=0.03). Negative values indicate carbon loss from leaves measured with a LiCOR- 6400.

Figure 9. The efficiency of photosystem II was measured before sunrise. We found no significant differences of treatment or site.



A
B
Temperature
Precipitation
Average Air Temperature (C)
Average Precipitation (mm)
24
22
20
18
16
14
12
10
8
6
4
400
300
200
100
0
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Figure 1

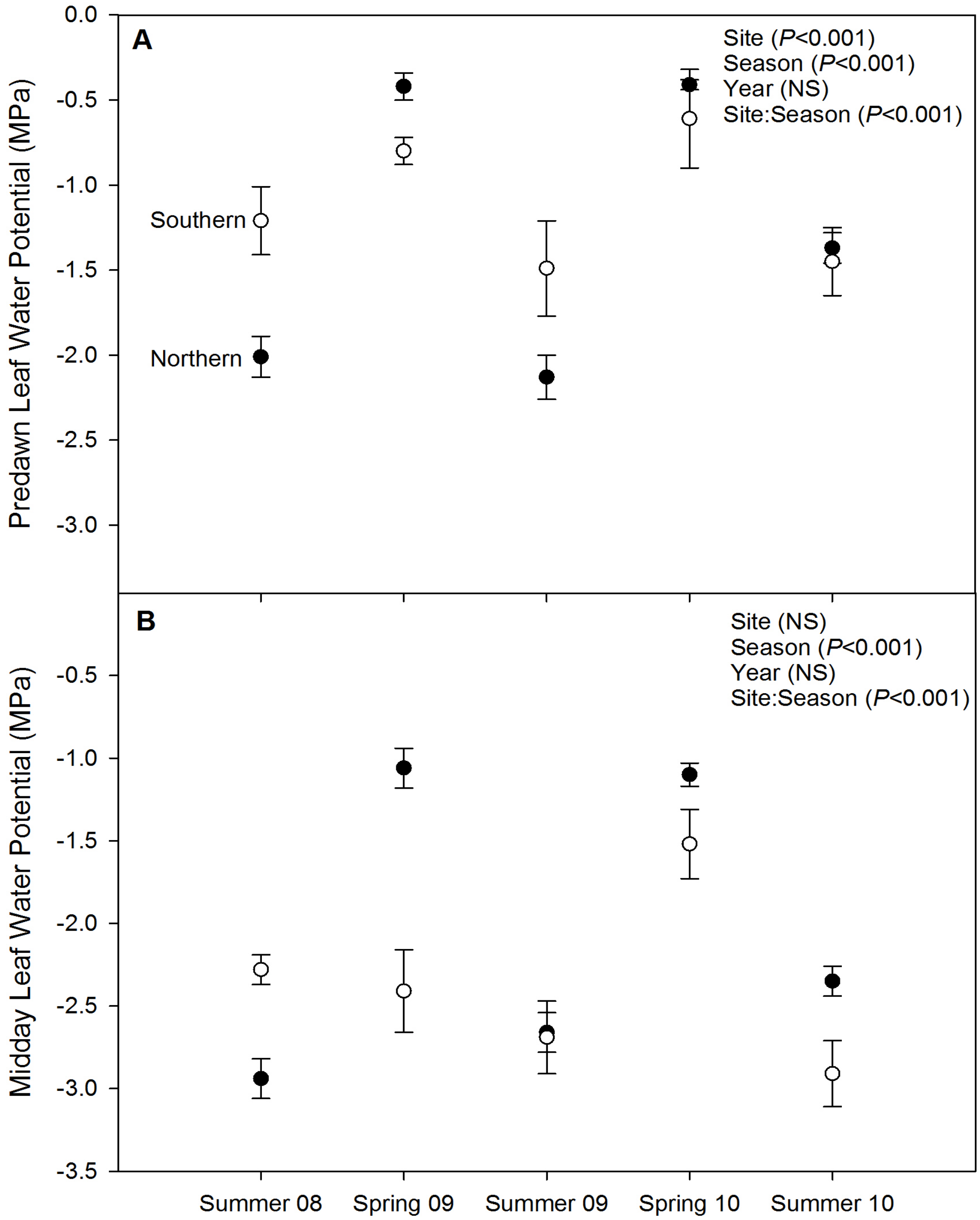

A
B
Predawn Leaf Water Potential (MPa)
Midday Leaf Water Potential (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
Southern
Northern
Site (*P*<0.001)
Season (*P*<0.001)
Year (NS)
Site:Season (*P*<0.001)
Site (NS)
Season (*P*<0.001)
Year (NS)
Site:Season (*P*<0.001)
Summer 08
Spring 09
Summer 09
Spring 10
Summer 10

Figure 2

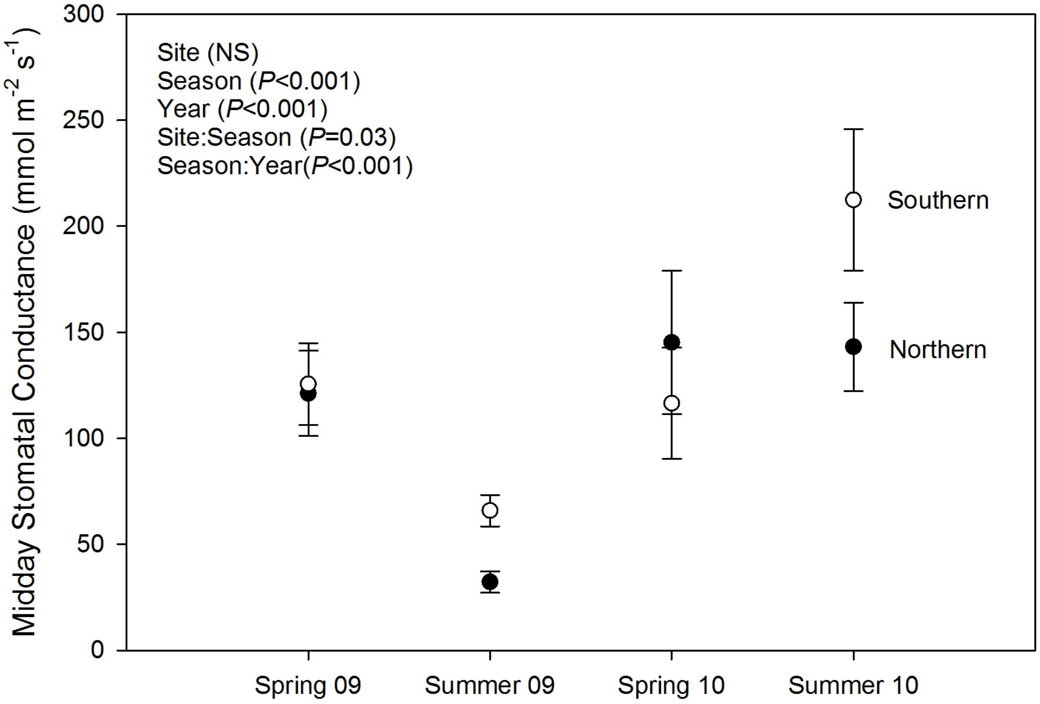
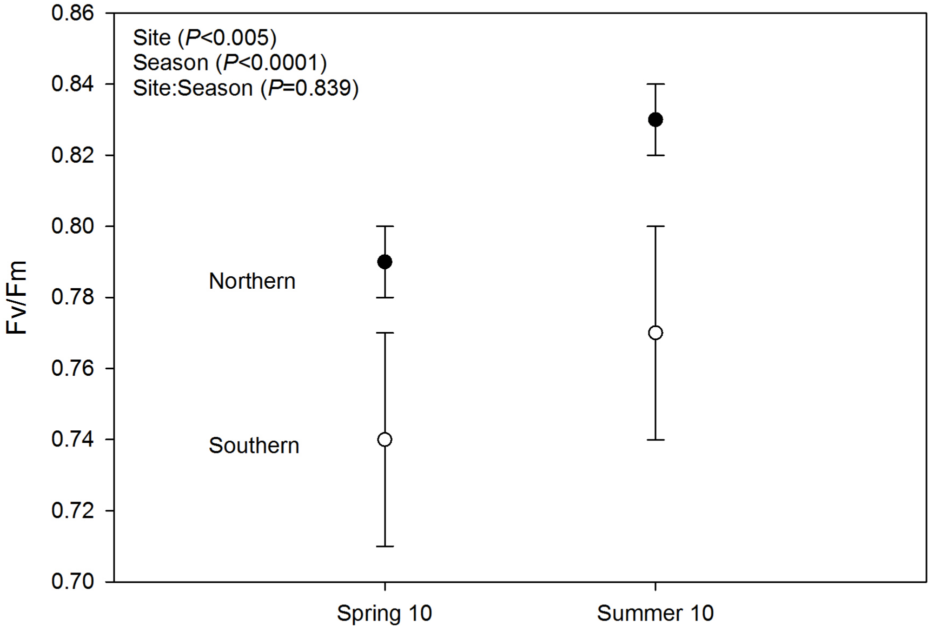

Midday Stomatal Conductance (mmol m-2 s-1)
300
250
200
150
100
50
0
Site (NS)
Season (P<0.001)
Year (P<0.001)
Site:Season (P=0.03)
Season:Year(P<0.001)
Southern
Northern
Spring 09
Summer 09
Spring 10
Summer 10

Figure 3



Site (P<0.005)
Season (P<0.0001)
Site:Season (P=0.839)
Northern
Southern
Fv/Fm
0.86
0.84
0.82
0.80
0.78
0.76
0.74
0.72
0.70
Spring 10
Summer 10

Figure 4

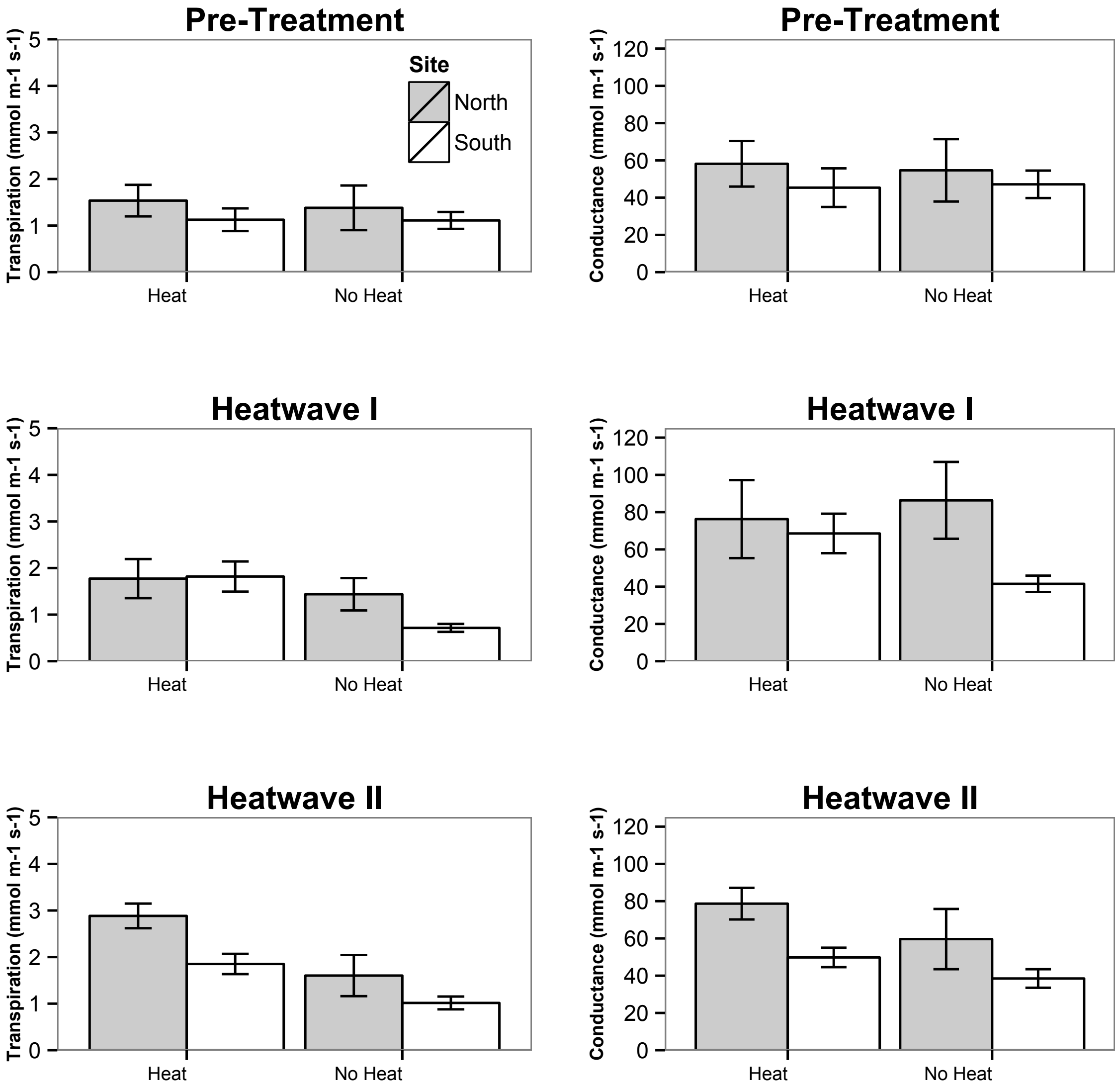

Pre-Treatment
Transpiration (mmol m-1 s-1)
Site
North
South
Heat
No Heat
Pre-Treatment
Conductance (mmol m-1 s-1)
Heatwave I
Heatwave II
0
1
2
3
4
5
20
40
60
80
100
120

Figure 5

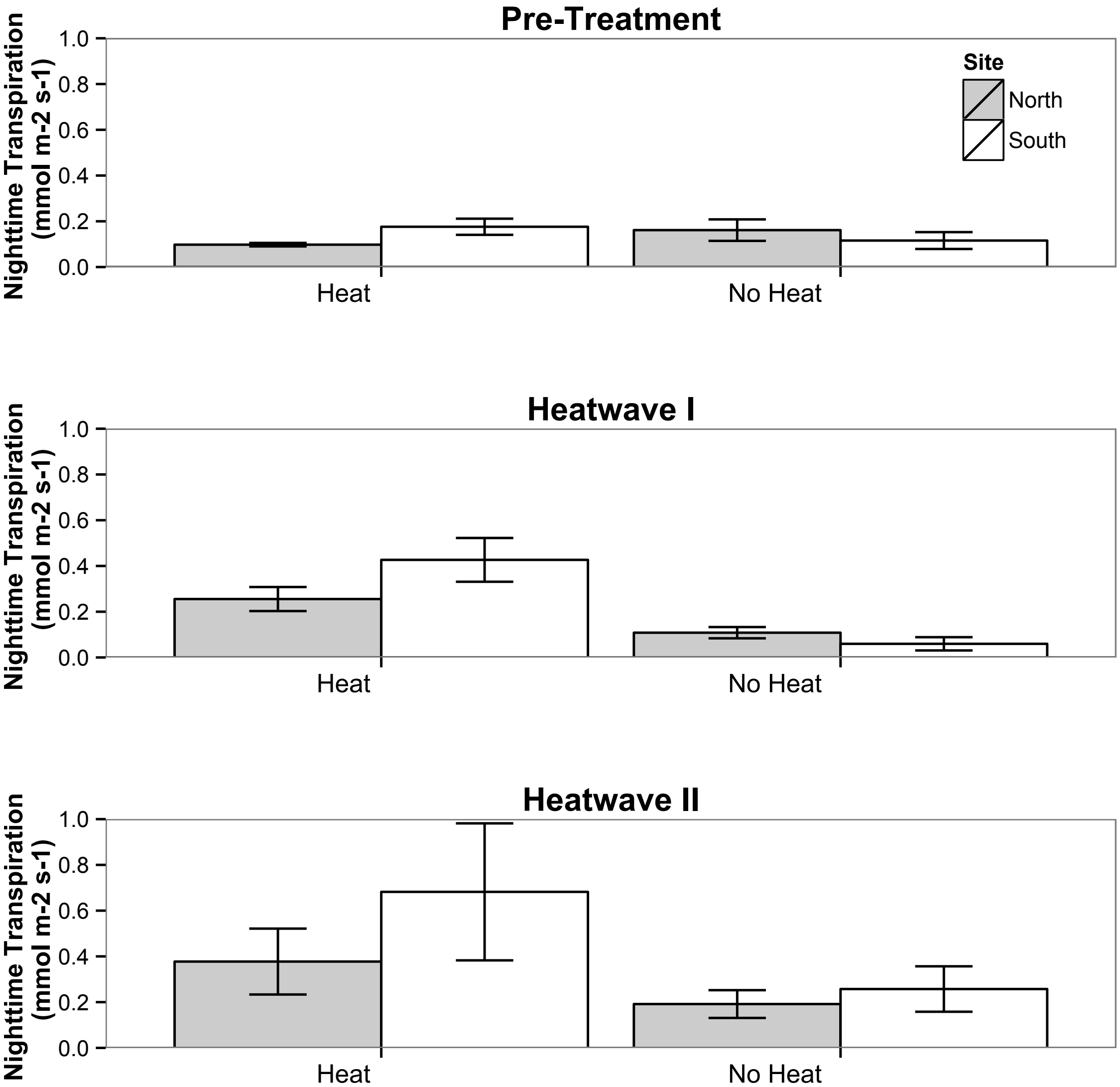

Pre-Treatment
Heatwave I
Heatwave II
Nighttime Transpiration (mmol m-2 s-1)
1.0
0.8
0.6
0.4
0.2
0.0
Heat
No Heat
Site
North
South

Figure 6

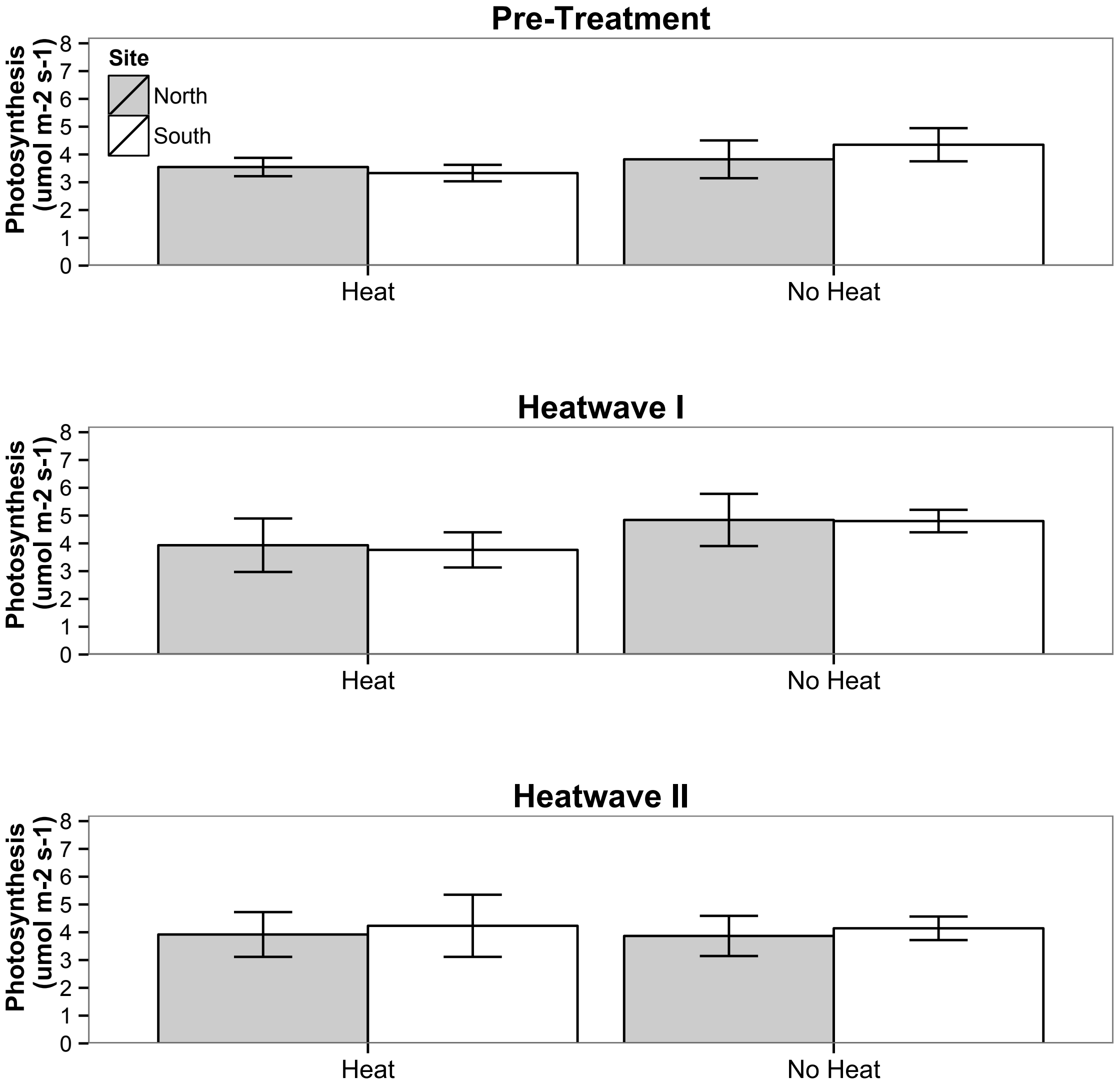

Pre-Treatment
Heatwave I
Heatwave II
Photosynthesis (umol m-2 s-1)
0
1
2
3
4
5
6
7
8
Site
North
South
Heat
No Heat

Figure 7

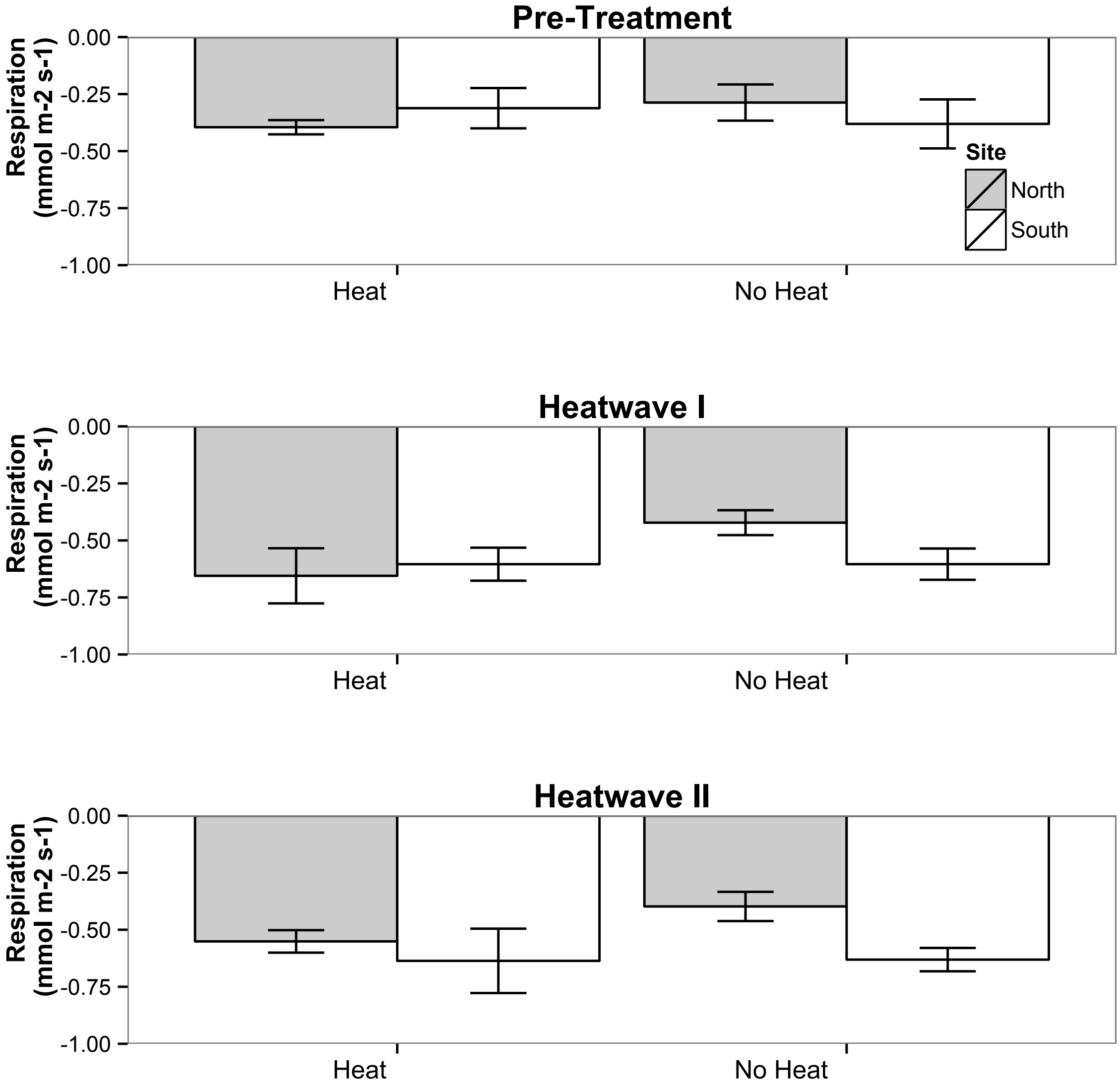

Pre-Treatment
Heatwave I
Heatwave II
Respiration (mmol m-2 s-1)
0.00
-0.25
-0.50
-0.75
-1.00
Heat
No Heat
Site
North
South

Figure 8

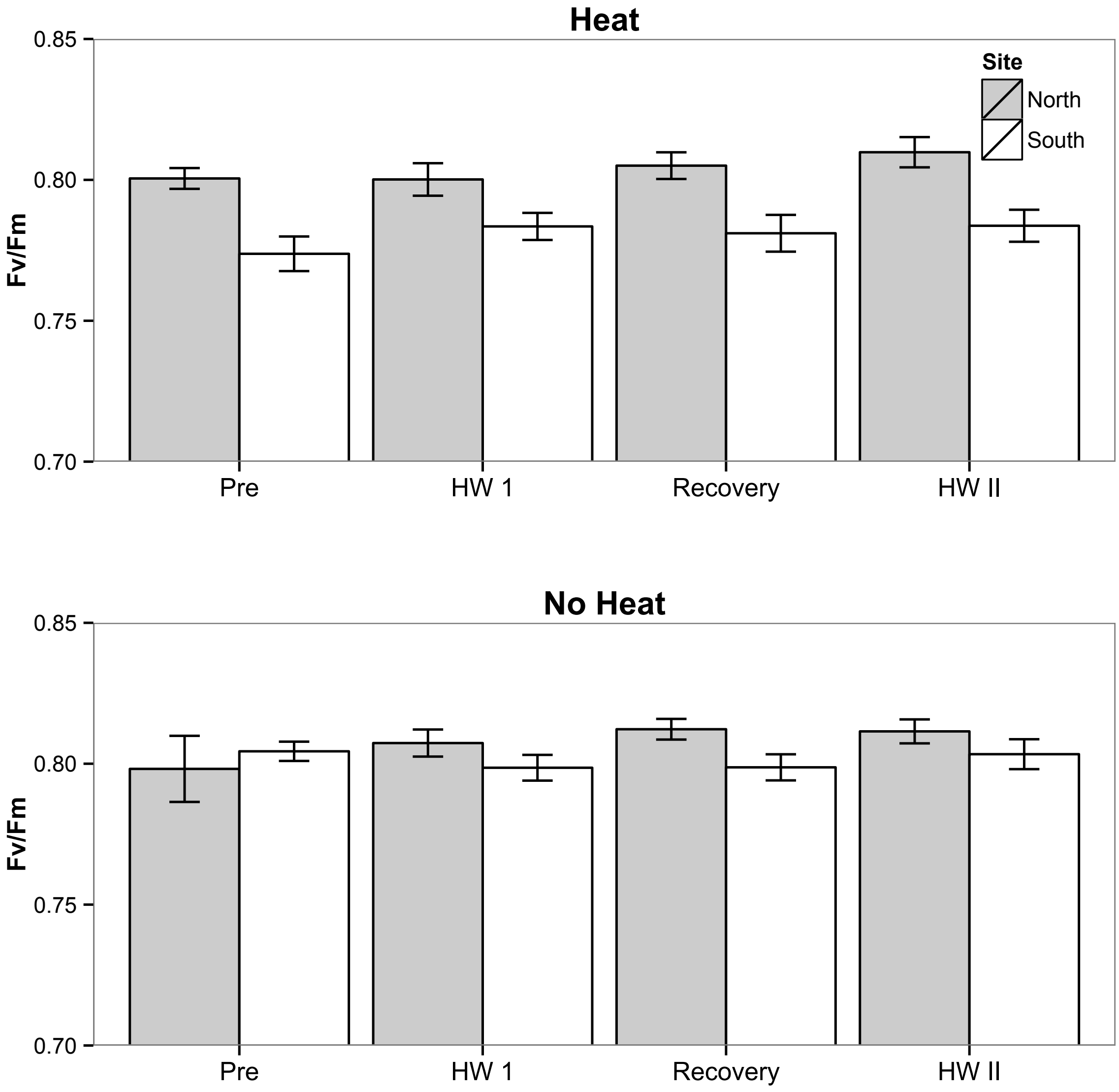
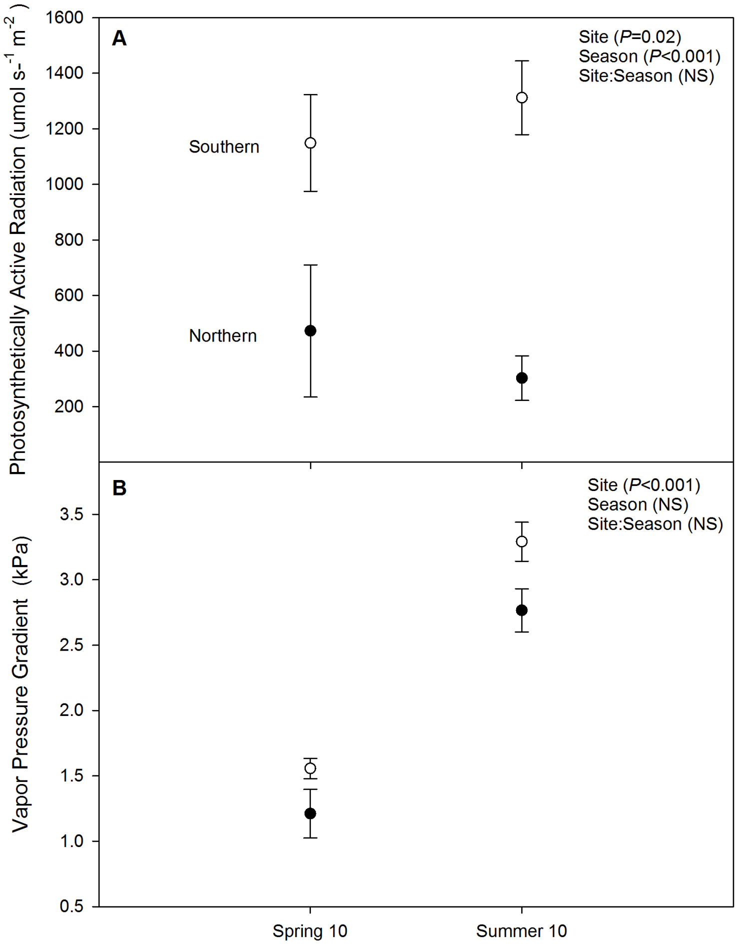
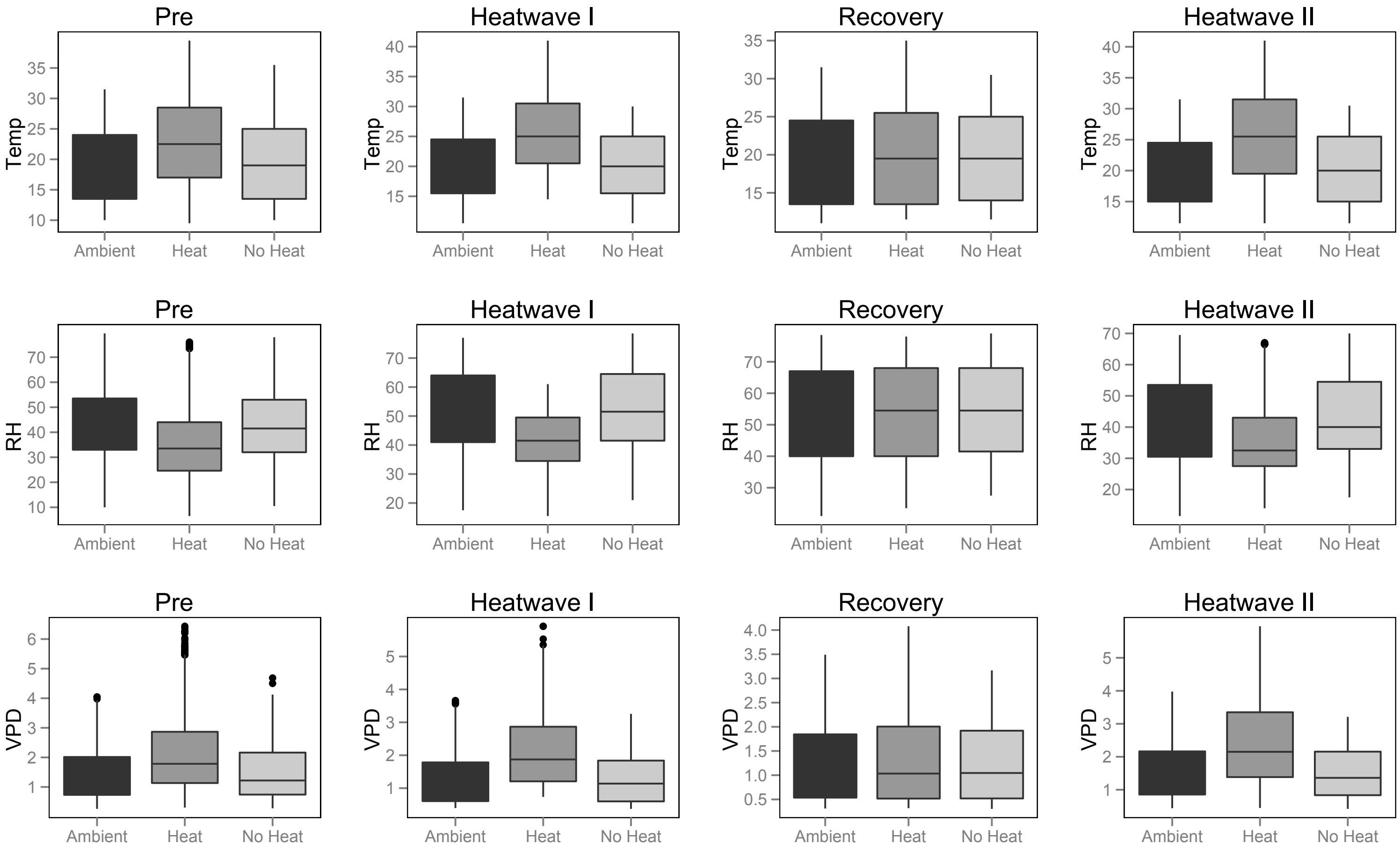

Heat
0.85
0.80
0.75
0.70
Fv/Fm
Site
North
South
Pre
HW 1
Recovery
HW II
No Heat
0.85
0.80
0.75
0.70
Fv/Fm
Pre
HW 1
Recovery
HW II

Figure 9

Supplementary Information



Supplementary Figure 1. Photosynthetically active radiation (PAR) and vapor pressure gradient from leaf to air at the southern (open circles) and northern (filled circles) sites. The southern site has much greater light availability than the northern site. The vapor pressure gradient from leaf to air was greater at the southern site in both spring and summer.



Supplementary Figure 2. Temperature, relative humidity, and vapor pressure deficit from the greenhouse experiment during the key measurement periods in ambient (black), heat (dark gray), and no heat (light gray) chambers. There were no differences between the three chamber types during the recovery period. The heat chambers had higher temperature and VPD during the heatwaves and lower relative humidity during the heatwaves

CHAPTER 2

Physiological responses of a California native shrub to a naturally occurring spring and fall heatwave

Abstract

Few studies have examined plant physiological responses in the field to naturally occurring heatwaves. In the future heatwaves are projected to increase in length, intensity and frequency. The severity of the impacts of heatwaves to plants likely depends on the severity of the heatwaves and the level of exposure. We examined the responses of resprouting and mature individuals of the California native shrub *Heteromeles arbutifolia* to naturally occurring spring and fall heatwaves. During both a spring and a fall heatwave, plants were able to maintain their normal water status on a diurnal basis as neither heatwave resulted in differences in water potential between pre-heatwave and heatwave days. During the spring heatwave stomatal conductance did not differ from pre-heatwave values; however, we saw that during the fall, when soil water availability was comparatively lower, stomatal conductance dropped during the heatwave. In water-limited conditions, such as the end of the dry season in a Mediterranean type climate, the restriction of water loss may be more important to survival than the transpirational cooling of leaves, assuming leaves stay below lethal temperatures. We found no evidence that either heatwave reduced carbon fixation or harmed the photosystem. One way that plants cope with high temperatures is by shifting the time of day of when they are most active. During the fall heatwave, electron transport rate (ETR) was higher in the morning compared to a pre-heatwave day and lower at the end of the day. We found that the resprouts in our study had higher conductance, transpiration, and ETR compared to mature shrubs; however, despite having higher physiological activity, resprouts and shrubs did not respond differently to the heatwaves we studied. Northern California populations of *H. arbutifolia* may be well adapted to cope with heatwaves of the magnitudes we examined. More research on multiple heatwaves in different years would be necessary to determine the stability of physiological processes response to heatwaves of varying frequency and intensity.

Introduction

Many plant physiological processes are controlled by variation in environmental conditions, from seasonal variation that drives phenological patterns to daily variation that drives rates of processes such as transpiration and photosynthesis. In a world of changing climate, changes in the magnitude and timing of fluctuations in environmental conditions is expected to alter plant functioning. Slight shifts in the seasonal timing of temperature changes have been shown to have relatively large effects on the timing of plant phenological patterns, such as earlier spring leaf flush (Menzel et al. 2006) and delayed autumn leaf senescence (Vitasse et al. 2009). Chronically elevated temperatures can alter baseline metabolic rates, resulting in increases in net photosynthesis and growth (Sage and Kubien 2007; Way and Oren 2010).

While numerous studies have examined plant physiological responses to chronically elevated temperatures (de Valpine and Harte 2001; Klein et al. 2004; Dukes et al. 2005) few studies have examined plant responses to heatwaves - short-term, intense increases in temperature that often occur every year (but see (Ameye et al. 2012)). A consensus definition of a heatwave is lacking, but heatwaves are generally considered to last for just a few days and are characterized by unseasonably hot, sustained temperatures or high temperature anomalies above that of preceding weeks (Ameye et al. 2012; Montero et al. 2010). We use a similar definition to others of at least 3-5 consecutive days with maximum temperatures at least 5°C higher than the average maximum temperatures for the same calendar days (Ameye et al. 2012; Hansen et al.; Parry et al. 2007; Tebaldi et al. 2006 ;Frich 2002). This definition emphasizes the importance of temperature relative to seasonal acclimation. For example, under this definition a winter heatwave could occur at very moderate temperatures, as long as they are above the long-term average. It is possible that a definition based on an absolute measure may be more relevant to temperature stress. For example, most proteins are known to start denaturing at temperatures above 42°C. Understanding the implications of heatwaves for plants is important because in the future heatwaves will likely increase in length, intensity and frequency across the globe (Coumou and Rahmstorf 2012; Lavell et al. 2012; Barriopedro et al. 2011; Mastrandrea et al. 2011; Orlowsky and Seneviratne 2011; Rahmstorf and Coumou 2011; Parry et al. 2007) . In the biodiverse region of California, the heatwave season (i.e. the period between the beginning of the year's first heatwave and end of the year's last heatwave) is predicted to increase length (Gershunov and Guirguis 2012; Hayhoe 2004), but it is unknown if the heatwave season will start earlier or end later. Furthermore, the timing of the heatwave season varies geographically throughout California, peaking in July in the central part of the state and in September along the coast (Gershunov and Guirguis 2012). This seasonal and geographic mosaic of heatwaves could have devastating consequences for plant species and community dynamics throughout the state if plants are exposed to an increase in the number and severity of heatwaves throughout the year.

One of the plant processes most likely to be affected by acute changes in temperature is photosynthesis. The potential of acclimation of photosynthesis to changes in temperature is well known (reviewed in (Berry and Bjorkman 1980)) but plant responses are highly variable among species (Gunderson et al. 2000). Seasonal acclimation of photosynthesis has been documented in the field as a shift in temperature optima (Ferrar et al. 1989; Slayter and Morrow 1977), an increase in photosynthetic rates in warmer months (Jurik et al. 1988) or both (Strain et al. 1976).

Heatwaves can affect both biochemical and biophysical aspects of photosynthesis and we examined both aspects in our study. At the biochemical level, heat stress occurs based on absolute definitions, where temperatures greater than 42°C can denature proteins and damage photosystem II by increasing fluidity of the thylakoid membrane (Rennenberg et al. 2006). Also, rates of photosynthesis can decline due to reductions in the efficiency of Rubsico at high temperatures (Rennenberg et al. 2006). At the biophysical level, high temperatures and low water availability often occur in concert and many of the physiological responses to low water availability, e.g. stomatal closure, are also likely to occur in response to high temperatures (Lambers et al. 1998). However, there is very little physiological research on plants experiencing a natural heatwave in the field, and even less research on the implications heatwaves in the field in relation to varying water availability. This study investigated the implications of naturally occurring wet-season spring and dry-season fall heatwaves on *Heteromeles arbutifolia*, an evergreen sclerophyllous shrub native to California.

California has a Mediterranean-type climate and plants experience dry and hot conditions every summer (Barbour et al. 2007). In the spring, water availability is high and temperatures are cool. This means that plants have plenty of water but they may not have acclimated to high temperatures. In the fall, water availability is low but plants may have acclimated to higher temperatures during the hot, dry summer. For example, plants may develop heat shock proteins to stabilize their membranes (Knight and Ackerly 2001), increase non-photochemical quenching to dissipate excess incoming energy (Müller et al.), or increase solute potential to maintain turgor in face of water stress (Davis and Mooney 1986).

In the seasonally dry Mediterranean-type climate of California an increase in heatwave intensity or frequency may also be accompanied by an increase in fire frequency (Davis and Michaelsen 1995). Like many plants native to fire-prone areas, *H. arbutifolia* has a resprouting strategy (Keeley et al. 2012). If fire frequency increases, then the population size structure may change such that more individuals in a population will have developed from recent resprouts. There is strong evidence that resprouting alters physiology compared to mature, non-resprouted shrubs (Goorman et al. 2011; Hernández et al. 2011; Paula and Ojeda 2009; Pratt et al. 2007; Ackerly 2004; Castell and Terradas 1994; Fleck et al. 1998; Schlesinger and Gill 1980). Therefore, resprouts and mature shrubs may differ in their responses to heatwaves.

In the present study we wanted (1) to characterize how plants respond to naturally occurring heatwaves in the field, (2) to determine whether the heatwave response varies with the season, and (3) to compare whether physiological adjustments due to resprouting influence the heatwave response. We examined how a spring and a fall heatwave affected carbon fixation and water use in shrubs and resprouts in the field. We hypothesized that in the spring, when water is abundant, the heatwave would not cause a reduction in physiological activity (i.e. gas exchange rates would stay the same or increase during the heatwave). Although in the spring plants may not have acclimated to high air temperatures, physiological functions would not be limited by water availability. In contrast, we hypothesized that in the fall, when water is limited, extremely high atmospheric vapor pressure deficits (VPD) and high temperatures during a heatwave would further reduce physiological activity below pre-heatwave levels (i.e. stomatal conductance or photosynthesis would decrease).

Materials and Methods

Species description

H. arbutifolia is a widespread California native shrub that occurs primarily from Humboldt and Shasta counties in northern California to the San Pedro Martir Mountains in Baja California, and scattered populations extend as far south as La Paz in Baja California (Morrow and Mooney 1974). It typically grows as a shrub 2-10 m high (Munz

1973) and has an intermediate rooting depth (Ackerly 2004; Davis and Mooney 1986). While *H. arbutifolia* experiences substantial water deficit in midsummer (Ackerly 2004; Calkin and Pearcy 1984a; Calkin and Pearcy 1984b; Davis and Mooney 1986) it tolerates drought by maintaining leaf turgor and gas exchange in the dry season through shifts in osmotic potential and the bulk modulus of elasticity (Davis and Mooney 1986). Unlike many chaparral shrubs, it has large leaves. It is insect pollinated and produces fruits that ripen and turn bright red in late fall. The fruits are primarily bird dispersed but are also consumed by mammals, such as bears and coyotes. Plants resprout vigorously after fire, and seedlings only establish in the understory during fire-free intervals (McMurray 1990).

Site description and sampling design

Quail Ridge Natural Reserve (38°49'04" N, 122°14'28" W) located in Napa County, CA lies on a peninsula in the Berryessa Reservoir on the eastern edge of the Coast Range. It is made up of a patchwork of vegetation types, including grasslands, chemise chaparral, and oak woodlands. Elevation ranges from 34 to 462 m above sea level and the average yearly rainfall is 62 cm (Boucher et al. 2004). The topography is complex and consists entirely of soils derived from Lower Cretaceous-Upper Jurassic marine mudstone, siltstone, sandstone, and conglomerate. Our site was located on a ridge top at a chaparral-grassland ecotone, above a chemise chaparral site that burned in the fall of 2005. In 2005 some of the plants at our site had their canopies mechanically removed to form a firebreak (other examples of non-fire resprouts include (Shelden and Sinclair 200: Fleck et al. 1996;). The site itself never burned in the 2005 fire. This left our site with interspersed mature, adult shrubs and resprouting shrubs. This allowed us to study the physiology of resprouted individuals, without the potentially confounding influence of fire history.

We sampled the plants in the spring of 2009 and the fall of 2010. During the spring sampling we sampled plants one day at the onset of a heatwave and one day during the heatwave (Figure 1, Table 1). In the fall we sampled plants on two days prior to the onset of a heatwave and two days during a heatwave (Figure 1, Table 1). On each sampling day we sampled the plants at approximately 2, 5, 7, and 9 hours after sunrise. On some of the sampling days we also sampled the plants before sunrise (see below for details on sampling protocols). We defined a heatwave as 3 or more consecutive days on which the daily maximum temperature exceeded the average daily maximum temperature by at least 5°C (Frich 2002).

Weather Data

Maximum daily temperature was obtained from Markely Cove station, located less than 2 km from our site (Western Regional Climate Center, http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca5360). Maximum daily temperatures have been collected at Markely Cove station since 1970. As a baseline we used a 30-year (1981-2010) maximum daily temperature (Figure 1). At our field site air temperature and relative humidity were logged every 30 minutes during spring 2009 (RH/TempLog Datalogger, Oakton Instruments, Vernon Hills, Il) and fall 2010 (EL-USB-2, Lascar Electronics, United Kingdom). Loggers were placed in plant canopies underneath a radiation shield made from a 4-in PVC drain cap. Vapor pressure deficit (VPD) was calculated as:

$$(1)\ VPD = e_{sat} - e_a = 0.61121^{\left(\frac{17.502T}{T+240.97}\right)} - \left[0.61121^{\left(\frac{17.502T}{T+240.97}\right)} * \frac{RH}{100}\right]$$

where T is air temperature and RH is relative humidity. Data were plotted continuously and as binned averages for each measurement period (Figures 2 and 3). The vapor pressure gradient (VPG) from leaf to air was calculated as:

$$(2)\ VPG = e_{sat} - e_a = 0.61121^{\left(\frac{17.502Tleaf}{Tleaf+240.97}\right)} - \left[0.61121^{\left(\frac{17.502Tair}{Tair+240.97}\right)} * \frac{RH}{100}\right]$$

where T_{leaf} is leaf temperature, T_{air} is air temperature and RH is relative humidity.

Gas exchange, leaf energy balance and fluorescence

During the spring sampling period we used a type-T (copper-constantan) fine wire thermocouple connected to a voltmeter that outputs temperature (Pro's Kit USA, Chesterfield, VA) and touched the thermocouple to the bottom of the leaf to measure instantaneous leaf temperature. In the fall we measured leaf temperature using the type-T thermocouple on the LI-COR 1600 porometer (LI-COR Biosciences, Lincoln, NE). We calculated the difference between leaf temperature and air temperature by subtracting the average air temperature from the loggers (see above) during the measurement period from the instantaneous leaf temperatures.

We measured stomatal conductance (g_s) on two leaves per plant at each time point using a steady state porometer (Decagon Devices, Pullman, WA). We then used the trapezoidal rule to approximate the definite integral of the diurnal conductance curve of each plant (MATLAB, MathWorks, Natick, MA). We estimated transpiration from our measurements of stomatal conductance and leaf to air vapor pressure deficit as:

$$(3)\ E = g_s\ * \frac{e_{sat} - e_a}{P}$$

where E is transpiration (mmol m^{-2} s^{-1}), g_s is stomatal conductance as measured above (mmol m^{-2} s^{-1}), e_{sat} is leaf internal vapor pressure (kPa) estimated from Tleaf, e_a is atmospheric water vapor pressure estimated from Tair (kPa), and P is the barometric pressure calculated from the elevation of our site (kPa). The leaf internal vapor pressure and atmospheric water vapor pressure were calculated as in equation 2.

We measured chlorophyll fluorescence and electron transport rate (ETR) on at least ten haphazardly selected leaves per plant at each time point (WALZ Mini-Pam Heinz Walz GmbH, Effeltrich, Germany). We sampled leaves at predawn to test for the potential quantum yield of photosystem II by measuring fluorescence with the light pulse on the fluorometer set to low and sampled leaves during the daytime to determine the ETR with the light pulse set to high.

Water Potential

During each sampling period we collected leaf water potential using a Scholander-type pressure chamber (Plant Moisture Stress, Albany, OR). We sampled plants before sunrise and at approximately 2, 5, 7, and 9 hours after sunrise. Leaves were excised with a razor blade, immediately wrapped in plastic wrap, put in a small ziptop bag, and placed in a dark cooler until they were measured. Leaves remained in the cooler before measurement for less than 90 minutes. Data gathered at the University of California, Berkeley campus using the same method indicated that there was no significant difference in water potential between adjacent leaves that were measured immediately or that were measured after sitting wrapped and in the dark cooler for 90 minutes (data not shown).

Carbon Isotope Discrimination

We sampled leaves from throughout the plant canopies at approximately 16:00 on each sampling day. Leaves were cut, placed in a manila coin envelope, immediately frozen in liquid nitrogen, and stored in a ziptop bag on ice until they were placed in a freezer. We then freeze-dried the samples, removed the petioles, and ground the lamina into a fine powder. Using a modified protocol (Brugnoli et al. 1988) we removed the leaf sugars by extracting 150 mg of ground leaf material in a 1:1 aqueous solution of Polyvinylpolypyrrolidone (PVPP, Sigma-Aldrich, Inc., St. Louis, MO). We removed amino acids from the solution with an ion exchange resin (Dowex 50WX8-100, Sigma Aldrich Inc., St. Louis, MO) and organic acids with an anion exchange resin (Dowex 1X2, Sigma Aldrich, Inc., St. Louis, MO). The final extract was freeze dried and analyzed with an elemental analyzer (ANCA/SLPDZ, Sercon, Cheshire, UK) coupled to a continuous flow isotope ratio mass spectrometer (Finnigan MAT, DeltaAPlus XL, Thermo Scientific, Bremen, Germany). The carbon isotope ratios (‰) were expressed relative to the international standard V-PDB. The carbon isotope discrimination was calculated as:

$$(4)\ \Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}/1000}$$

where Δ is discrimination, $\delta^{13}C_{air}$ is the isotope ratio of the air and $\delta^{13}C_{leaf}$ is the isotope ratio of the leaf sugars. The $\delta^{13}C_{air}$ of the atmosphere was determined as the average value of the source air at the field site from three sampling times (4/2009, 5/2010, 11/2010). We sampled the source air by filling a 60 ml syringe with air and slowly expunging it into a 12ml Extetainer vial (Labco Limited, Buckinghamshire, England). We took quality control samples by sampling from a compressed air tank of know isotope value that was transported to the field. All quality control samples were treated like the unknown samples. We analyzed the samples within 48 hours of collection using a Thermo Gas Bench II coupled to a Thermo Delta Plus XL continuous flow isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). We calculated carbon isotope ratios of the air relative to the international standard V-PDB. There were no significant differences in $\delta^{13}C_{air}$ between the sampling dates (P=0.06, one way repeated measures ANOVA performed in Sigma Plot, Systat Software, Inc.) and therefore we used the average carbon isotope value in our calculation of discrimination (-8.59 ‰).

Statistics

For variables that did not have a time of day component (i.e. discrimination, daily conductance, F_v/F_m) we used a univariate split-plot design to perform repeated measures standard least squares regression using the REML method (JMP, SAS Institute). The whole plot effect was Type (Shrub/Resprout), the individual plants were nested within Type and set up as a random effect to test the between subject effects. The within subject effects were tested with the Date and a Type x Date interaction. We performed post hoc Tukey HSD on effects that were significant in the overall model. When there was no significant difference between shrubs and resprouts the data were pooled and the model was rerun. For variables with a time of day component (i.e. environmental data, transpiration, electron transport rate and water potential) we nested time of day within Date and performed the analyses as above.

Results

Environmental Data

Long-term 30-year average data from the Markley Cove weather station showed that in the spring our pre-heatwave day was 1.1°C above the 5°C cutoff, and that the heatwave day was 7.9°C above the 30-year average. In fact, the day of the spring heatwave (Julian Day 110) had not seen higher maximum temperatures for 70 years (data from Markley Cove weather station, see Methods). During the fall heatwave we had two pre-heatwave sampling dates, but only a subset of the measurements were made on the second pre-heatwave day (Table 1). Both days (Julian Day 259 and 266) were below the 30-year mean (Figure 1). The first heatwave day (Julian Day 270) was right at the 5°C threshold and the second heatwave day (Julian day 273), where only a subset of the measurements were made, was well above the 5°C threshold (Figure 1). At our site, increases in canopy air temperature occurred along with increases in VPD during both heatwaves, however only the fall heatwave had a dramatic decrease in RH (Figure 2).

When we binned the VPD data by the measurement period, VPD was significantly greater during the spring heatwave day compared to the pre-heatwave day only at the timepoint 9 hours after sunrise (Figure 3A). VPD was greater at all afternoon time points during the fall heatwave compared to the pre-heatwave day (Figure 3B). When we binned canopy air temperature by the measurement period we found that during the spring heatwave air temperatures were higher compared to pre-heatwave days during the afternoon (Figure 3C). The spring heatwave canopy air temperatures were greater than pre-heatwave temperatures during the sampling periods 7 and 9 hours after sunrise, but there was no significant differences in canopy air temperature before sunrise or 2 and 5 hours after sunrise. The patterns were more complex during the fall heatwave (Figure 3D). During the fall heatwave the temperature was always lowest during the post-heatwave sampling days for all time periods. The canopy air temperature was significantly greater during the heatwave before sunrise, hours after sunrise and 7 hours after sunrise. At 2 hours after sunrise and 9 hours after sunrise there were no significant differences between canopy air temperature during fall heatwave and pre-heatwave days. A similar pattern was found for VPG, where VPG was significantly different during the spring heatwave (Figure 3E) only during the last daily time point, but it was different in the morning and early afternoon timepoints during the fall heatwave (Figure 3F).

Leaf energy balance

During both heatwaves leaf temperatures were significantly different between the pre-heatwave and heatwave days, but the patterns varied with season (Figure 4A). During the spring heatwave leaf temperature was significantly *lower* during the heatwave compared to a pre-heatwave day at 9 hours after sunrise. There were no sigifincant differences between pre-heatwave and heatwave leaf temperatures at the other timepoints in the spring. During the fall heatwave leaf temperature was significantly *higher* during the heatwave compared to a pre-heatwave day at 2, 5, and 7 hours after sunrise, but there were no differences 9 hours after sunrise (Figure 4B).

There were no significant differences in leaf-air temperature at any timepoint between pre-heatwave and heatwave days during the fall heatwave period (Figure 5B). During the spring heatwave (Figure 5A) the greatests difference between leaf and air temperature was found in the afternoon (i.e. 9 hours after sunrise). During the heatwave leaf temperature was lower than air temperature at these timepoints. During both seasons leaf temperature was less than air temperature 5, 7, and 9 hours after sunrise timepoints (i.e. negative values).

Water relations

Overall, resprouts had higher stomatal conductance than shrubs (*P*=0.0003). There was no difference in conductance between pre-heatwave and heatwave days during the spring, but during the fall there were differences between sampling dates (Figure 6); however, there was not an overall trend that conductance was lower during the heatwave (See Table 1 for sampling scheme). The first pre-heatwave day (9/16) had higher g_s than the second heatwave day (9/30), but not the first heatwave day (9/27). There were no differences between the second pre-heatwave day (9/23) and either of the heatwave days. Overall, calculated transpiration was higher in resprouts than in shrubs (*P*<0.0001; Figure 7). When we examined the relationship between stomatal conductance and water potential we saw that some of our plants experienced stomatal closure in the fall, but that closure occurred at low water potential both before and during the heatwave (Supplemental Figure 1). There was no significant difference in transpiration before and during the heatwave and there were no significant differences when comparing pre-heatwave and heatwave days at specific time points.

Leaf water potential of mature shrubs and resprouts did not differ significantly (Figure 8). There were no significant differences between the pre-heatwave and heatwave days. Plants had significantly lower water potential in the fall compared to the spring, but plants were not measured in the same water year. There were no significant differences in leaf water potential between pre-heatwave and heatwave days at each time point.

Carbon fixation

We found no significant differences in discrimination among shrubs and resprouts, sampling day, or seasons (Figure 9). There were no significant differences between shrubs and resprouts or between different sampling dates in potential photosystem II efficiency (F_v/Fm), which was only measured in the fall (Figure 10). Electron transport rate was only measured during the fall and the results varied by time of day (Figure 11). At 2 hours after sunrise ETR was higher during the heatwave than both the pre-heatwave and post-heatwave days. At 5 hours after sunrise, the heatwave day had higher ETR than the pre-heatwave day. Nine hours after sunrise the heatwave day had lower ETR than the pre-heatwave. Resprouts had higher ETR at 5 hours after sunrise on all days, but 7 hours after sunrise resprouts were higher than shrubs only on pre-heatwave and heatwave days.

Discussion

Despite our general hypothesis that fall heatwaves would more negatively impact plant processes than spring heatwaves, we found that physiological processes in *H. arbutifolia* were largely unaffected by heatwaves regardless of whether soil water availability was high in the spring or limited in the fall. A strong heatwave response in the field for drought tolerant shrub species such as *H. arbutifolia* may require heatwaves of larger magnitude or heatwaves coupled with severe drought. These extreme heatwaves or combinations of extreme heatwaves and drought are rare and therefore difficult to capture in the field; however, it is just such events that are likely to be the most important to understand to predict plant vulnerabilities as extreme events become more common in the future.

Heatwaves can affect photosynthesis a biochemical or biophysical levels. On a biochemical level, we found no evidence that the fall heatwave disrupted photosystem II integrity (i.e. Fv/Fm was not different), but we did see differences in ETR during the heatwave. We only measured ETR in the fall and we found that during the heatwave ETR was higher in the morning and lower in the afternoon (Figure 11). Photosynthesis is one of the most thermosensitive plant processes and heat stress can damage or reduce efficiency of photosynthetic machinery (Wang et al. 2008; Berry and Bjorkman 1980). Especially when leaf temperature reaches 40 to 45°C. The research on temperature responses of photosynthesis has focused on changes in mean temperature, not changes in extreme excursions from the mean such as the heatwaves we study here. In our study canopy air temperatures and leaf temperatures never reached 45°C (Figures 2 and 4).

By using carbon isotope discrimination of the most recently fixed carbon, soluble leaf sugars, we were able to assess the daily-integrated carbon fixation (Fleck et al. 1996; G D Farquhar et al. 1989). Since discrimination is negatively correlated with water use efficiency (i.e. mol CO_2/mol H_2O) we expected that if water loss increased, as was likely in the spring heatwave, we would see water use efficiency decrease and discrimination increase. However, we saw no effect of the heatwave on discrimination during either heatwave. This suggests that *H. arbutifolia* has mechanisms in place to help it deal with high temperatures. More research would be needed to determine if these mechanisms are the same during heatwaves in the spring and fall. We also saw no differences between seasons in discrimination (i.e. spring vs fall). However, it is important to note that the spring and fall heatwaves did not occur in the same year. When we compared discrimination of soluble leaf sugars on a monthly basis within a water year we have found seasonal effects, with higher discrimination in the spring than the fall (see Chapter 3). Temperature response curves performed on field-plants in the spring and fall would provide information on the temperature optimum for photosynthesis, which may be different in different seasons. "Heat hardening" due to successive, hot days can increase thermotolerance, which may be further reinforced by the additive effects of strong solar irradiation and seasonal water deficit (Larcher 2000). To determine thresholds of thermotolerance, the best strategy is to further sample the plants *in situ* during the hottest period in the summer, as suggested for Mediterranean maquis plants (Larcher 2003).

During the fall heatwave we found additional evidence that photosystem II remained intact and fully functional during the heatwave. We measured the potential efficiency of PSII photochemistry at predawn (Fv/Fm). Evergreen angiosperms not experiencing stress to PSII have an average value of 0.83 (Demmig and Bjorkman 1987), and we found no reductions in Fv/Fm. While Fv/Fm provides an indication of the potential efficiency of PSII, ETR approximates the actual photochemical activity (Larcher 2000; Genty et al. 1989); however, this number involves a number of assumptions if reflectance and absorptance are not properly measured on the leaves (Baker 2008; Maxwell and Johnson 2000) One strategy to limit potential artifacts is to sample the exact same leaves throughout the day (Maxwell and Johnson 2000); however we used a random set of leaves that were in the sun.

Plants may cope with high temperatures by shifting the time of day of when they are most active. For example, throughout the course of a drought, the timing of the daily peak in photosynthesis shifted from a single peak curve centered on midday, first to a double peaked curve with midday depression, and then to a single peaked curve with the highest rates in the early morning and closure the rest of the day (Mooney et al. 1975). While we did not measure photosynthesis on an instantaneous basis throughout the day, our ETR support a similar trend. We found that during the fall heatwave plants shifted the time of day when ETR was the highest. Compared to pre-heatwave days, ETR was higher in the morning during the heatwave (2 and 5 hours after sunrise) and lower at the end of the day (9 hours after sunrise, Figure 11). If photosynthesis itself increased in the morning during the heatwave, then the differences were not great enough to be evident on the daily time scale of our discrimination values. Instantaneous gas exchange measurements (e.g. with a LiCor 6400) would be one way to examine this further, but it is difficult to make accurate measurements in the field under extremely high temperatures (personal observation).

In addition to the effects of heatwaves on the biochemical aspects of physiology, they can also affect plants on a biophysical level. On a biophysical level, the plants in this study were able to maintain their normal water status on a diurnal basis as neither heatwave resulted in differences in average water potential between pre-heatwave and heatwave days (Figure 8). This suggests that regardless of the soil water availability, in the ranges we measured, the heatwave had no effect on the leaf water status and that the plants were able to maintain physiological activity during the heatwave. It is possible that plants would have responded differently to a heatwave of greater magnitude or a heatwave of similar magnitude but with lower soil water availability (e.g. in a drought year). Average minimum leaf water potentials never reached -4 MPa, which is when others have seen stomatal closure in *H. arbutifolia* (Miller and Poole 1979); however as water potentials approached -4MPa in individual plants stomatal conductance decreased and in some individuals stomatal closure occurred during and before the heatwave

(Supplemental Figure 1). Our data suggest that the heatwaves were below the temperature threshold for damage to the photosynthetic machinery. In other words, while the plants experienced a heatwave from a weather perspective, the heatwaves we measured were within the operating range for *H. arbutifolia.* Compared to the September 2009 predawn water potentials (shrubs = -2.5 ±0.4 MPa and resprouts =-2.2 ± 0.5 MPa for resprouts; data not shown), the pre-dawn water potentials in September 2010 were less negative, indicating that the soil was relatively hydrated for September (Figure 8). One reason the water potentials in fall 2010 may be higher than might be expected is that the 2010 water year (September 2009-May 2010) had higher rainfall than the 2009 water year (742.4 mm rain compared to 545.3 mm rain; data from Markley Cove station, see Methods for details).

H. arbutifolia is known to tolerate low water potentials, and it may take a severe drought to cause catastrophic xylem failure. For example, *H. arbutifolia* growing in southern California lost fifty percent of its conductivity at -6.2 MPa (Jacobsen et al. 2007), a water potential well below any we have measured at our site (personal observation, see Chapter Three) or others have measured in other northern California sites (Ackerly 2004; Mooney and Chu 1974). Osmotic adjustment is one mechanism that allows *H. arbutifolia* to tolerate low water potentials. *H. arbutifolia* plants have been shown to increase solute concentrations from progressive cellular dehydration during drought as well as accumulate osmotica (Calkin and Pearcy 1984b; Davis and Mooney 1986). Another mechanism that allows *H. arbutifolia* to tolerate low water potentials is that it has small vessel pits and narrow but abundant vessels (Jarbeau et al. 1995) that provide high safety from xylem failure while reducing conductivity. *H. arbutifolia* also has multiple mechanisms that would protect the photosynthetic apparatus. At nearby Stebbins Cold Canyon Reserve, others found structural photoprotection with steep leaf angles up to 71.3 (±16.3) degrees (Valladares and Pearcy 1999). Steep leaf angles and self-shading would result in reductions in carbon gain but would minimize photoinhibition (Valladares and Pearcy 1999). *H. arbutifolia* also has thick leaves and a thick cuticle (Balsamo et al. 2003).

While we did not see differences in water potential between pre-heatwave and heatwave days in either season, there were differences in stomatal conductance (Figure 6). Stomatal conductance is a key physiological parameter that connects plant water and carbon use. There was no effect of the heatwave on stomatal conductance in the spring, when soil water availability was high; however, we saw that during the fall, when soil water availability was comparatively lower, stomatal conductance dropped during the heatwave (Figure 6). Shrubs that are tolerant of water stress close their stomata at relatively low water potentials.

It is well known that in water-limited habitats stomatal sensitivity to air humidity can change during the season as soil water availability declines (Schulze et al. 1974). When soil water availability is high, increases in stomatal conductance and transpiration lead to evaporative cooling. This prevents leaves from overheating, avoiding damage to the photosystem and lowering leaf temperature to a range more suitable for photosynthesis (Valladares and Pearcy 2008; Gates 1968). Without transpiration and evaporative cooling, leaf temperatures can exceed air temperatures even when air temperature is below lethal values (Valladares and Pearcy 2008; Lovelock et al. 1992; Comstock and Mahall 1985). In our study saw evidence of evaporative cooling (Figure 5); however, we are unsure what caused such a dramatic difference between leaf and air temperatures because stomatal conductance, leaf water potential and transpiration were all unaffected by the heatwave. One possibility is that cooling was due to an increase in boundary layer conductance caused by an increase in windspeed in the late afternoon of the heatwave, although we did not measure wind speed at our site.

In water limited conditions, such as the end of the dry season in a Mediterranean type climate, limiting water loss may be more important to survival than the transpirational cooling of leaves which results in favorable conditions for photosynthesis, assuming leaves stay below lethal temperatures. In this study stomatal conductance declined prevents evaporative cooling (Figure 5, Figure 6). Transpiration and water potential remained the same before and during the fall heatwave, when the vapor pressure gradient from leaf to air was higher, suggesting that stomata were closing to maintain constant water status (Figure 7).

Shrubs and resprouts

Plants in Mediterranean-type ecosystems have a number of traits adapted to fire, including resprouting (Keeley et al. 2012). When the tops of obligate or facultative sprouters (Pratt et al. 2012) are killed, shoots are produced from belowground meristems fueled by non-structural carbohydrate reserves (Paula and Ojeda 2009; Bell and Pate 1996; Kozlowski 1992). Resprouts use these reserves to maintain and regrow, but they also need to refill their starch reserves (Goorman et al. 2011; Paula and Ojeda 2009), and there is extensive evidence that resprouts have high photosynthetic rates, higher stomatal conductance, and higher transpiration rates than mature shrubs (Wildy et al. 2004; Fleck et al. 1998; Fleck et al. 1996; Schlesinger and Gill 1980). Another explanation for enhanced activity in resprouts is that they temporarily revert to a pre-reproductive or juvenile stage (Iwasa and Kubo 1997). In juvenile woody plants rates of photosynthesis, stomatal conductance, and transpiration are typically higher than in adults (Goorman et al. 2011; Niinemets 2010; Thomas and Winner 2002; Bond 2000).

Numerous studies have examined the physiology of resprouts in comparison to nearby mature shrubs or seedlings. Typically, these studies compare resprouts and shrubs or seedlings following a recent disturbance, and very few look at longer-term implications of the disturbance on resprout physiology. We examined resprouts that had their canopy removed 4 years prior to the onset of the study. Many previous studies have shown that resprouts have higher photosynthetic rates, stomatal conductance, growth, and transpiration. Despite the longer interval between disturbance and our sampling period compared to previous studies, we found that the resprouts continued to have higher conductance, transpiration, and ETR compared to mature shrubs (Figures 6, 7, 11). Counter to our initial hypothesis that higher physiological activity in resprouts would result in greater reductions due to the heatwave, we found that neither shrubs nor resprouts were dramatically affected by the two heatwaves we studied. It is possible that younger resprouts would have a different response to a heatwave. If they have a relatively large root area for a given shoot area they may be buffered from the heatwave if evaporative cooling results from the increased access to soil water resources.

Conclusions

This study suggests that northern California populations of *H. arbutifolia* may be well adapted to cope with heatwaves of the magnitudes we examined. We found when seasonal water availability was lower during a fall heatwave, plants reduced stomatal conductance, but when water availability was higher during a spring heatwave stomatal conductance was unaffected by the heatwave. We found that resprouts tend to have higher ETR and rates of conductance and transpiration, yet these differences did not result in shrubs and resprouts responding differently to either heatwave. In an experimental study on *H. arbutifolia* seedlings (Valladares and Pearcy 2008) plants experiencing water stress closed their stomata, reducing transpiration, CO_2 exchange and transpirational cooling. The seedlings down regulated photosynthesis, sacrificing carbon gain for water conservation and photoprotection via steep leaf angles and higher energy dissipation (Valladares and Pearcy 2008). *H. arbutifolia* experiences high water deficits (Davis and Mooney 1986), reaching midday water potential of -3.5MPa in this study, and its highly efficient use of water helped it avoid water stress (Davis and Mooney 1986). Water conservation strategies, such as stomatal closure, are probably triggered by hot, dry atmospheric conditions before severe water or heat stress occurs. Such a preventative strategy would be useful for plants growing in environments with multiple co-stressors (i.e. water and heat) and variable resources and will likely help *H. arbutifolia* cope with an increase in extreme heat events. It is likely that for chaparral shrubs such as *H. arbutifolia* an absolute definition of a heatwave may be more informative than the relative definition we used. For example, sustained temperatures above 42°C may result in strong declines in photosynthesis, transpiration and increases in stress parameters, such as Fv/Fm. Further research on multiple spring and fall heatwaves in wet and dry years would help determine how interannual variability in water availability may influence the responses of *H. arbutifolia* to increases in the frequency, duration and severity of heatwaves.

Figures:

Figure 1. The maximum daily temperature (black filled circles), 30-year average maximum daily temperature (white filled circles) and the 30-year average plus 5°C (grey filled circles). Data are from Markely Cove station (Western Regional Climate Center, http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca5360) plotted by day of the year (DOY). Sampling dates before the heatwave are labelled with blue circles and sampling dates during the heatwave are labelled with red circles. During the spring heatwave (A), the sampling day of April 20th was the hottest day in 70 years on that day of the month. During the fall heatwave (B), the sampling day of September 30th was the hottest day in 43 years on that day of the month. Missing values are due to missing data from the weather station.

Figure 2. Plant canopy air temperature and humidity were logged every 30 minutes during the measurement periods. Panel A shows the canopy vapor pressure defict (VPD), relative humidty (RH) and air temperature in spring 2009. Panel B shows the same in fall 2010. During both heatwaves the air temperature and VPD increased but the RH dropped much more dramatically in the fall.

Figure 3. We binned the vapor pressure deficit (A and B), canopy air temperature (C and D) and vapor pressure gradient from leaf to air (E and F), in the spring and the fall respectively. to emcompase the measurement period at each timepoint. Measurements were taken before sunrise, and approximately 2, 5, 7, and 9 hours after sunrise on each sampling day. Stars indicate timpoints within a season when the pre-heatwave and heatwave day are significantly different from each other.

Figure 4. Leaf temperature was measured with a thermcouple touching the bottom of the leaf. Leaf temperature in spring (A) was lower during the heatwave in the late afternoon, which was the time of day with greatest different in temperature between pre-heatwave and heatwave days. Fall (B) leaf temp was greater during the heatwave in the middle part of the day. Measurements were taken before sunrise, and approximately 2, 5, 7, and 9 hours after sunrise on each sampling day. Stars indicate timpoints within a season when the pre-heatwave and heatwave day are significantly different from each other.

Figure 5. Leaf-air temperature was greater in the spring (A) on pre-heatwave days, but there were no differences between pre-heatwave and heatwave days in the fall (B). In the spring leaf-air temperature was closer to zero on the pre-heatwave days. During the heatwave leaf-air temperature was negative. Therefore leaf temperature was less than air temperature during the spring heatwave but not the fall heatwave. Measurements were taken before sunrise, and approximately 2, 5, 7, and 9 hours after sunrise on each sampling day. Stars indicate timpoints within a season when the pre-heatwave and heatwave day are significantly different from each other.

Figure 6. Daily integrated conductance was caclulated by performing a trapezoidal integration under the a diurnal curve of condtance for plants measured approximately 2,

5, 7, and 9 hours after sunrise. There were no significant differences in conductance between pre-heatwave and heatwave days in the spring. A post-hoc Tukey HSD test showed that in the fall, conductance was significantly lower during the heatwave days compared to the pre-heatwave days.

Figure 7. Transpiration was modeled from conductance and vapor pressure gradient for plants measured approximately 2, 5, 7, and 9 hours after sunrise. There were no significant differences in transpiration between pre-heatwave and heatwave days in either season.

Figure 8. The top panel shows diurnal time courses of leaf water potential during spring on pre-heatwave and heatwave days for resprouts (A) and shrubs (B). The bottom panel shows diurnal time courses of leaf water potential during fall on a pre-heatwave da and heatwave day. We found no significant differences of shrubs and resprouts or differences between pre-heatwave and heatwave days within a season.

Figure 9. Discrimination was measured on soluable leaf sugars before and during the heatwave in spring and fall. We found no significant differences between shrubs and resprouts or of day of the study.

Figure 10. Predawn Fv/Fm was measured in the fall before and during and the heatwave. There were no significant difference between shrubs and resprouts and Fv/Fm was not lower during the heatwave.

Figure 11. Electron transport rate was measured before and during the fall heatwave over a dirunal time course. Resprouts had higher ETR at the 5 hour timepoint on all days but at the 7 hour timepoint resprouts were higher than shrubs on pre-heatwave and heatwave days.

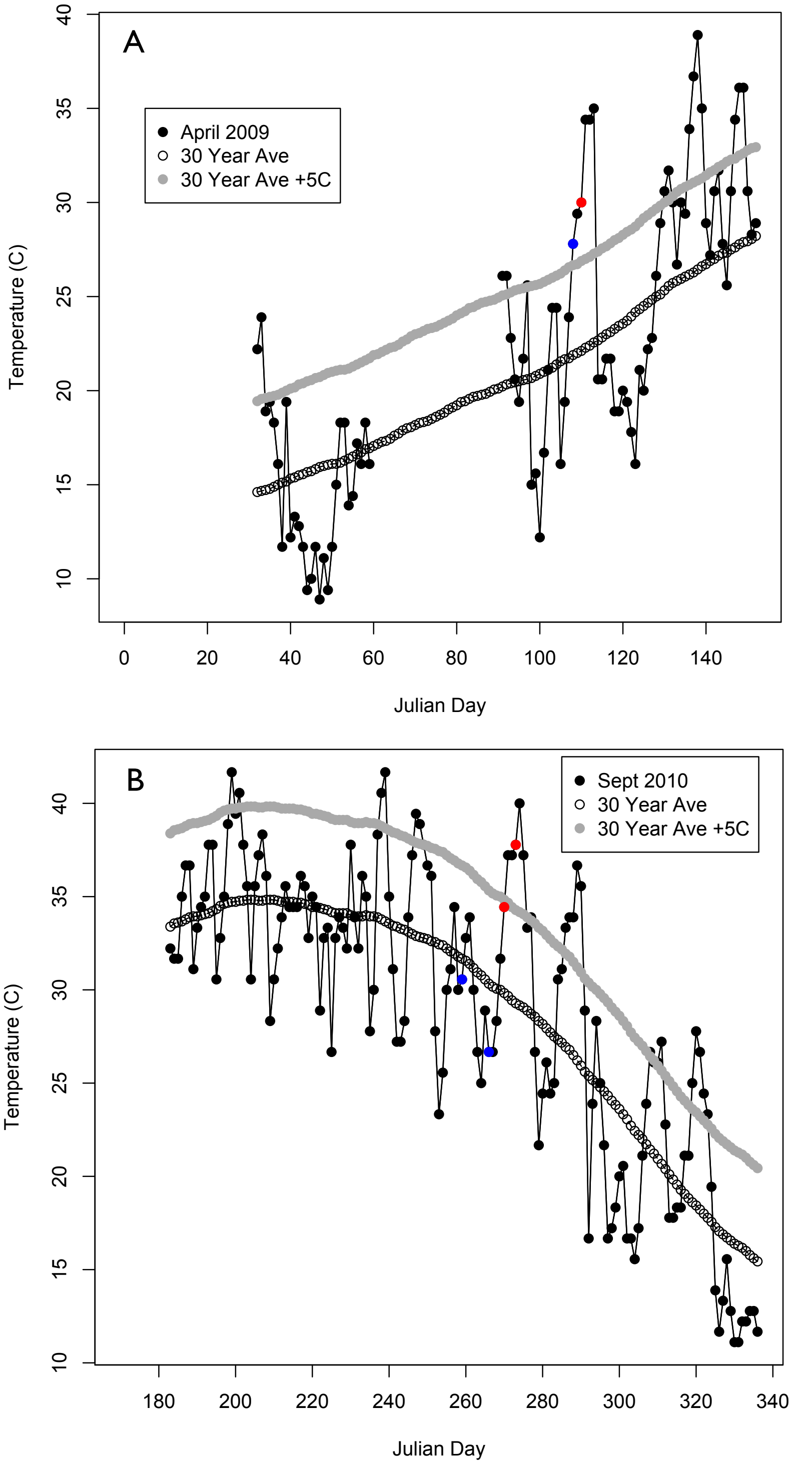

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Figure 1

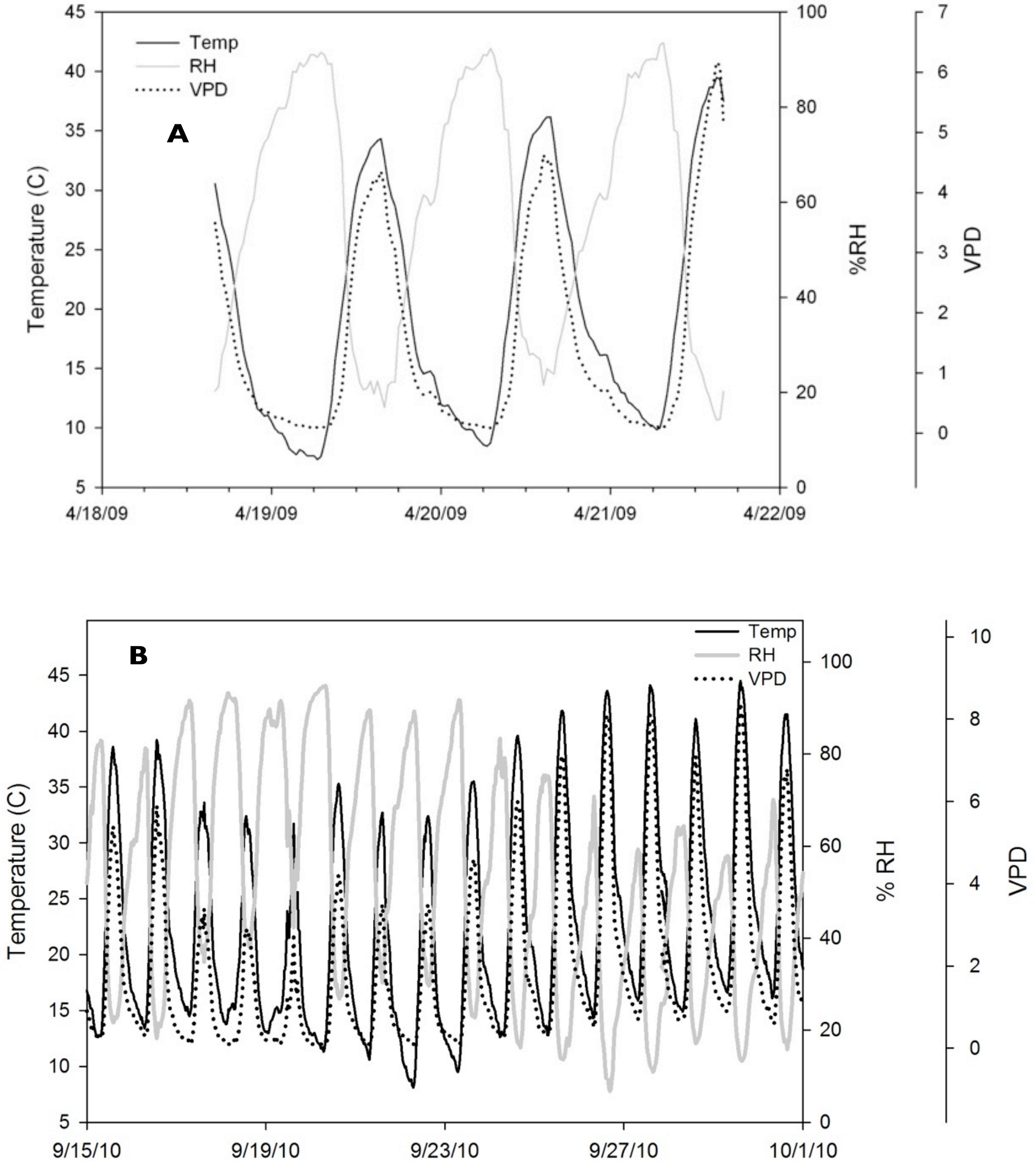
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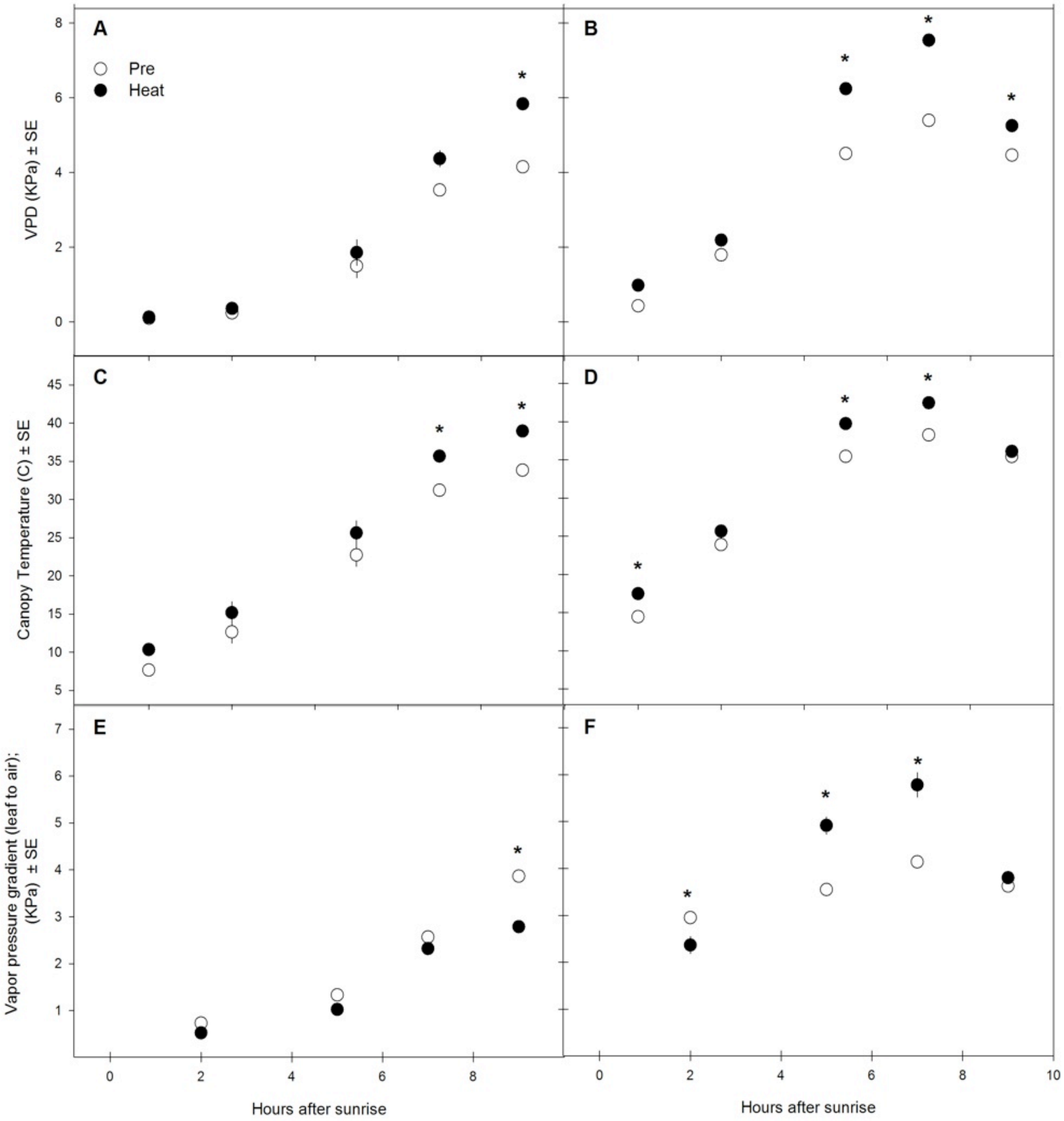
Figure 2



A
B
C
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○ Pre
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VPD (KPa) ± SE
Canopy Temperature (C) ± SE
Vapor pressure gradient (leaf to air); (KPa) ± SE
Hours after sunrise
Hours after sunrise
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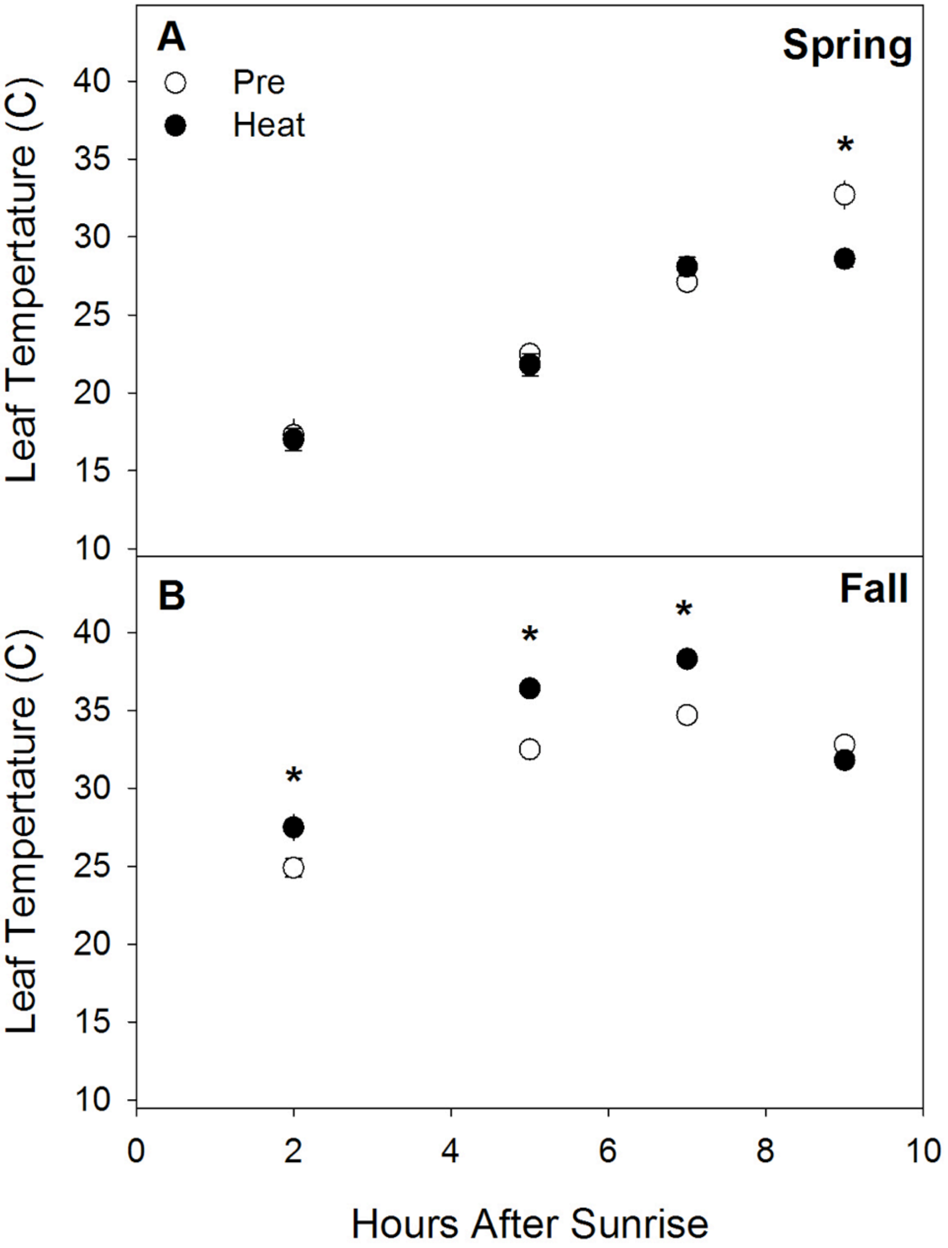
*

Figure 3



A
Spring
B
Fall
○ Pre
● Heat
Leaf Tempertature (C)
10
15
20
25
30
35
40
*
0
2
4
6
8
10
Hours After Sunrise

Figure 4

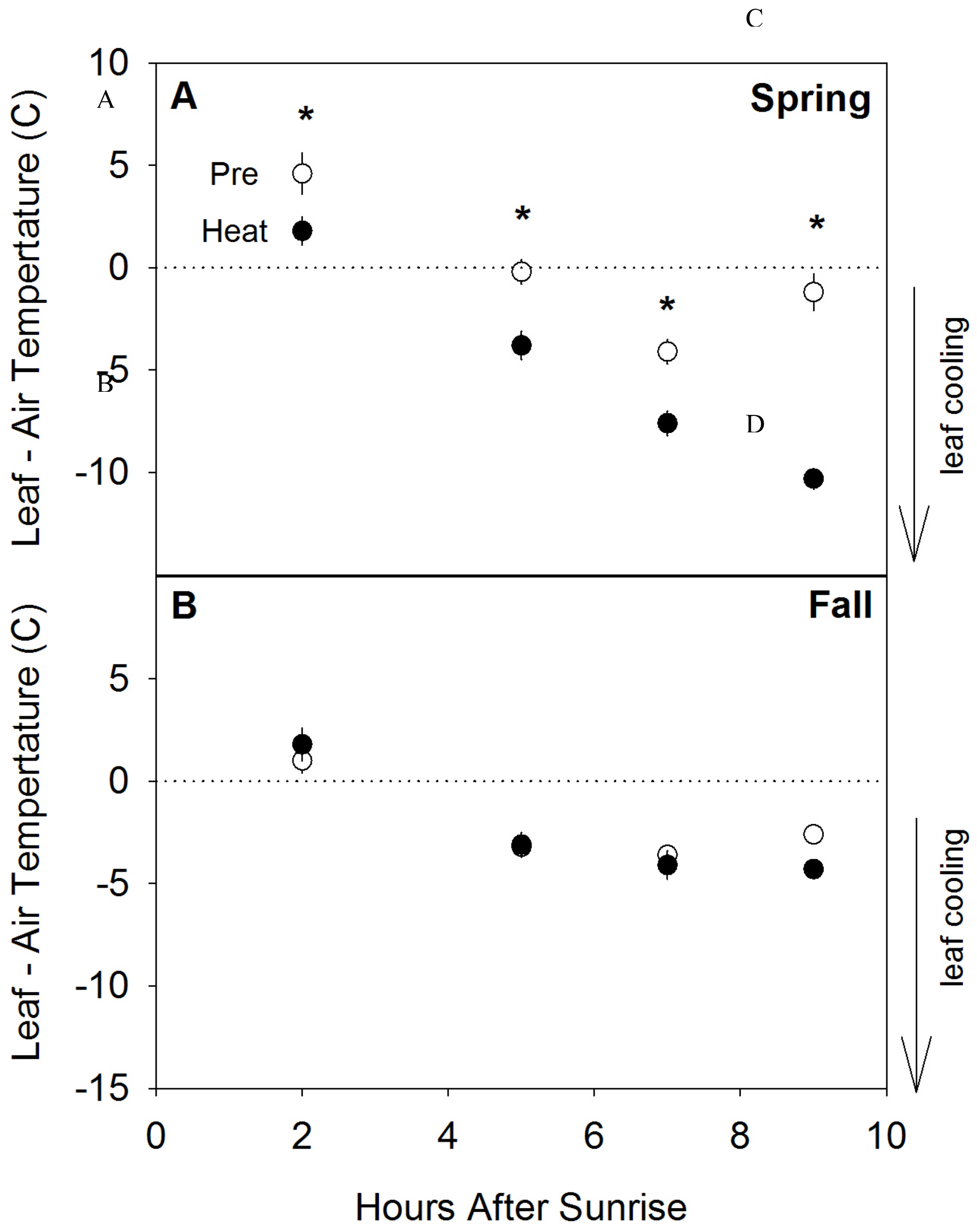
C
A
B
D
A
Spring
B
Fall
Pre
Heat
*
*
*
*
Leaf - Air Tempertature (C)
Leaf - Air Tempertature (C)
10
5
0
-5
-10
5
0
-5
-10
-15
0
2
4
6
8
10
Hours After Sunrise
leaf cooling
leaf cooling

Figure 5

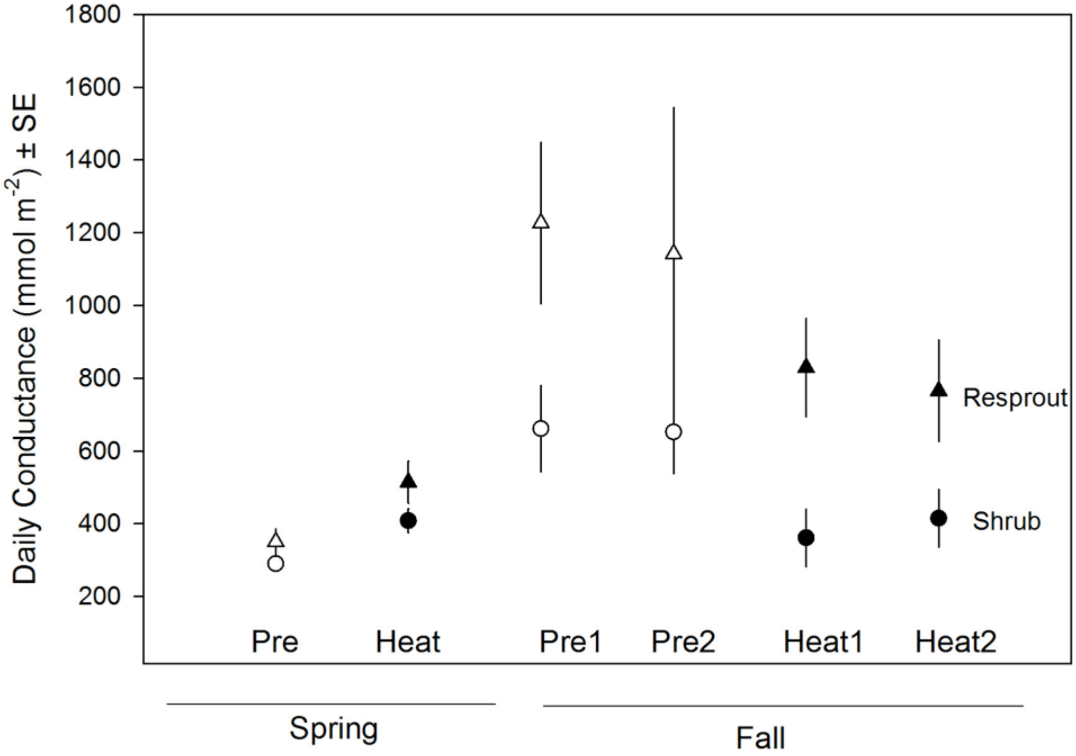
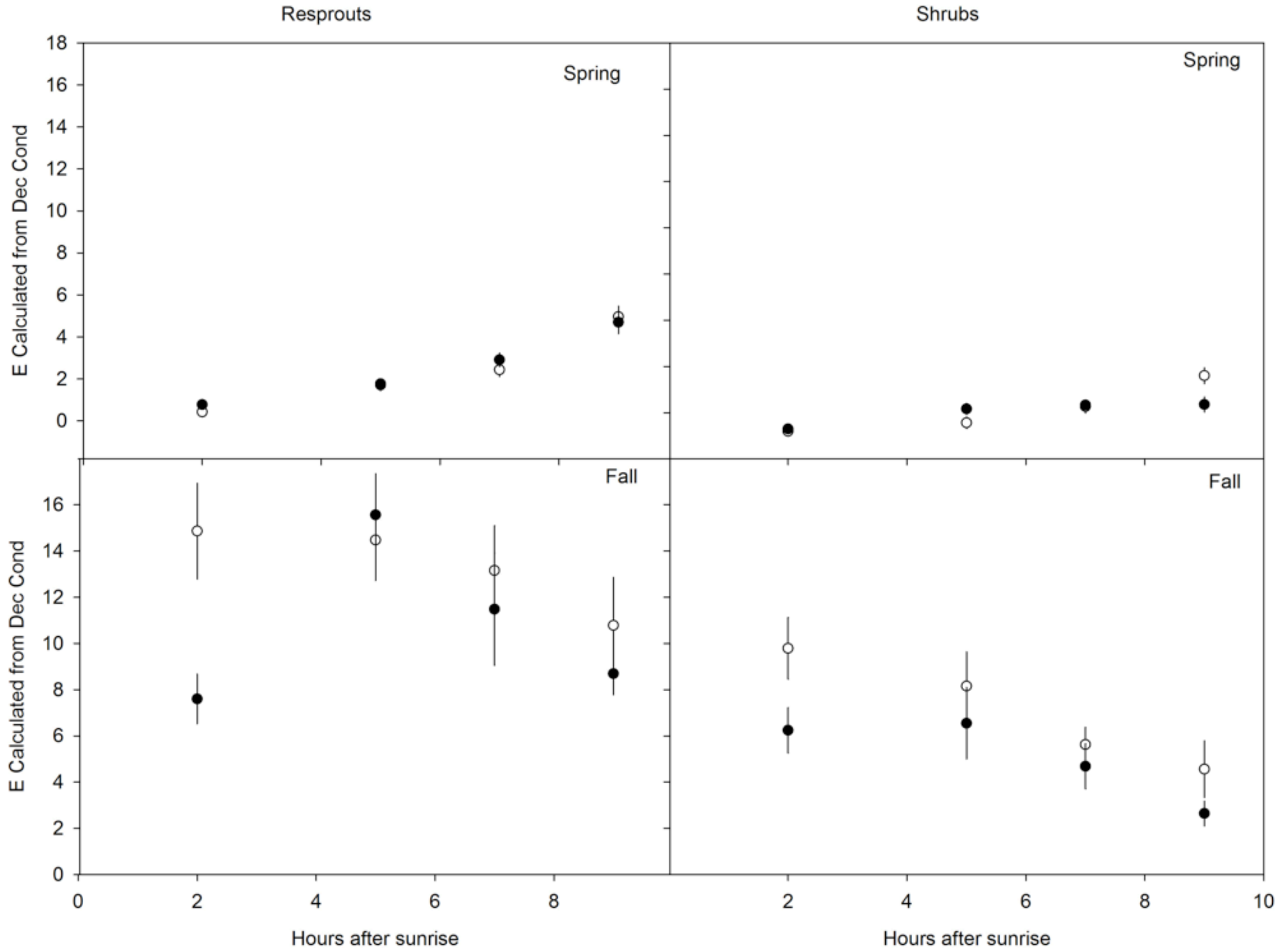

Daily Conductance (mmol m-2) ± SE
1800
1600
1400
1200
1000
800
600
400
200
Pre
Heat
Pre1
Pre2
Heat1
Heat2
Resprout
Shrub
Spring
Fall

Figure 6



Resprouts
Shrubs
Spring
Spring
Fall
Fall
E Calculated from Dec Cond
E Calculated from Dec Cond
18
16
14
12
10
8
6
4
2
0
16
14
12
10
8
6
4
2
0
0
2
4
6
8
2
4
6
8
10
Hours after sunrise
Hours after sunrise

Figure 7

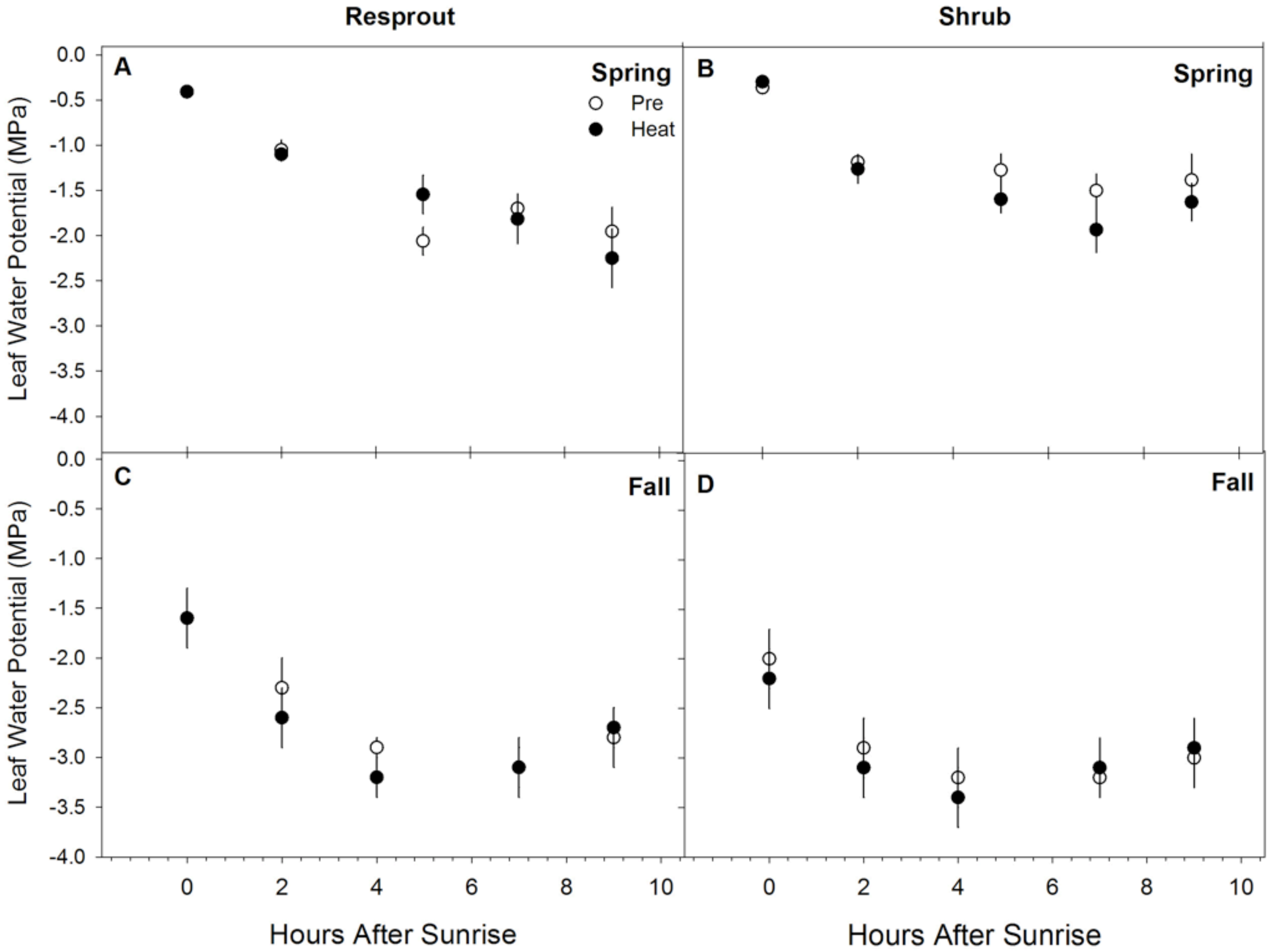

Resprout
Shrub
A
Spring
○ Pre
● Heat
B
Spring
C
Fall
D
Fall
Leaf Water Potential (MPa)
Leaf Water Potential (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
0
2
4
6
8
10
Hours After Sunrise
Hours After Sunrise

Figure 8

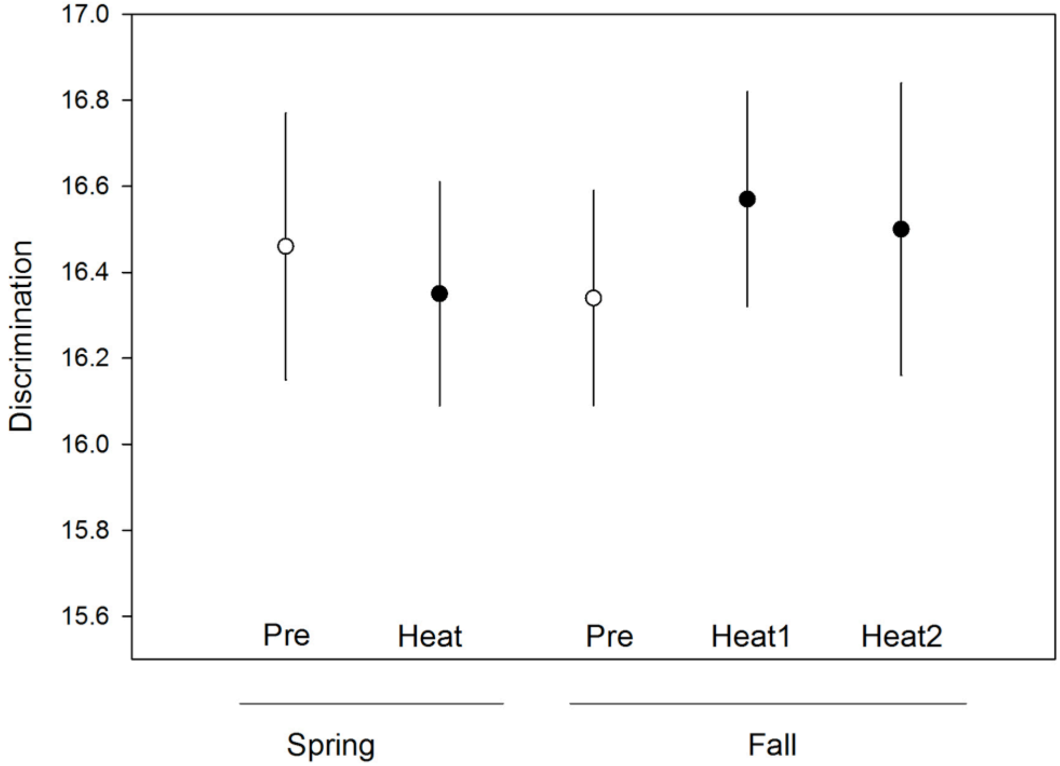

Discrimination
17.0
16.8
16.6
16.4
16.2
16.0
15.8
15.6
Pre
Heat
Pre
Heat1
Heat2
Spring
Fall

Figure 9

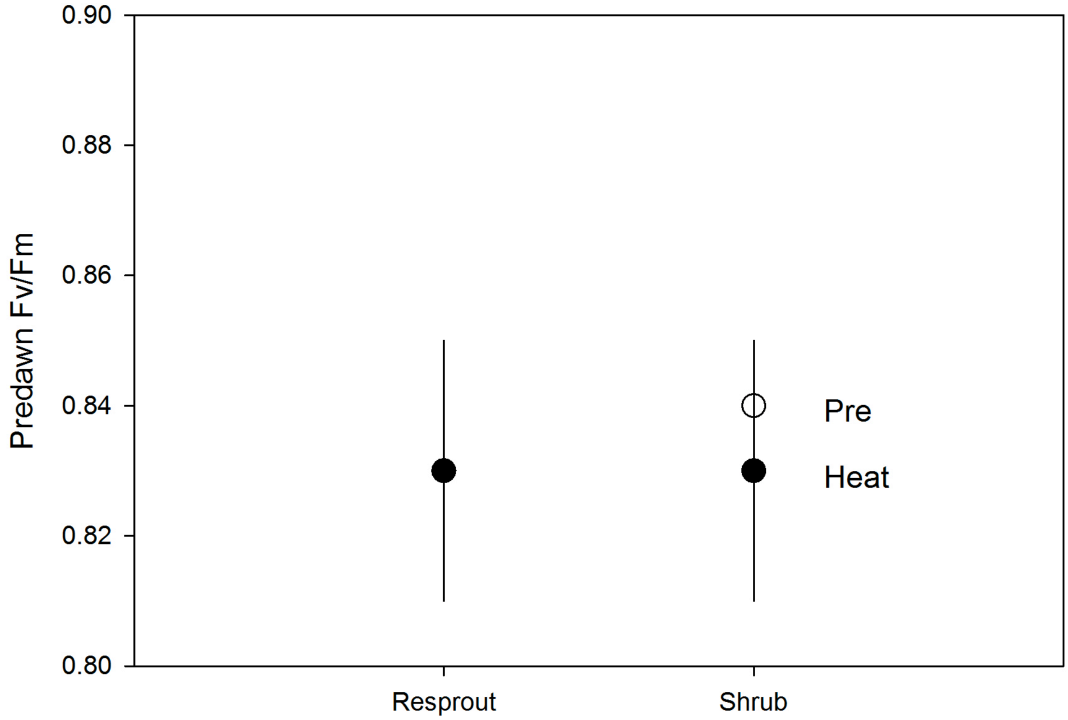


Figure 10

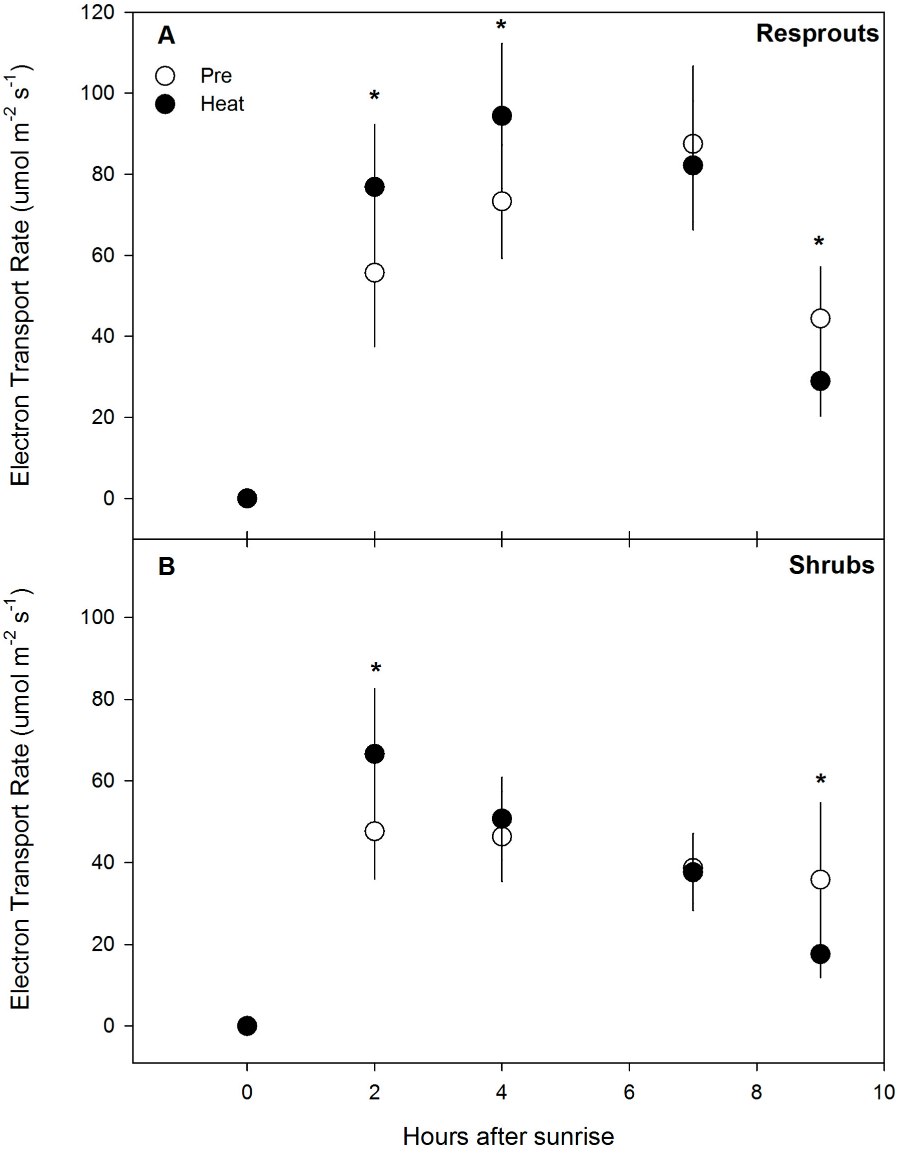
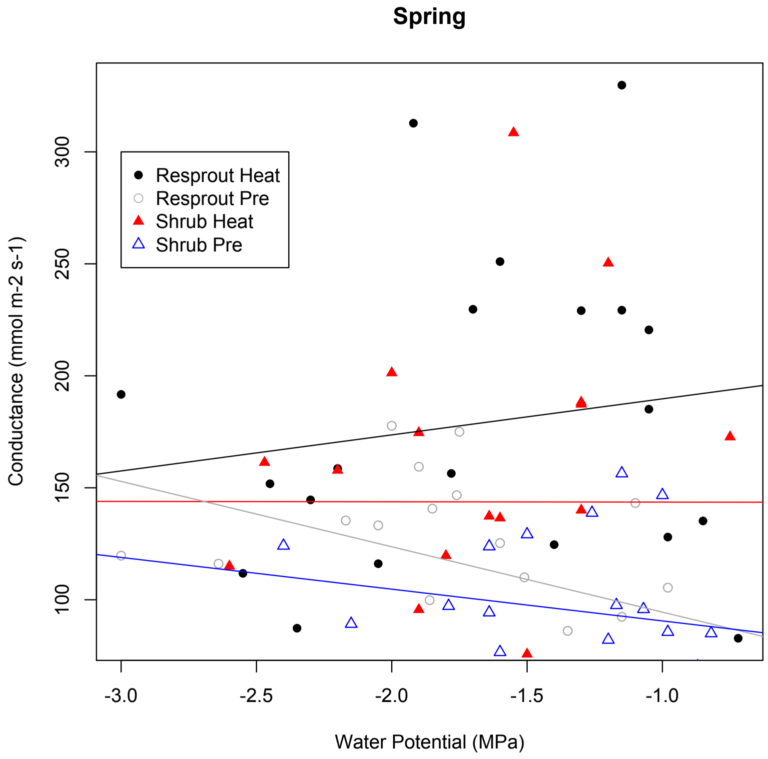
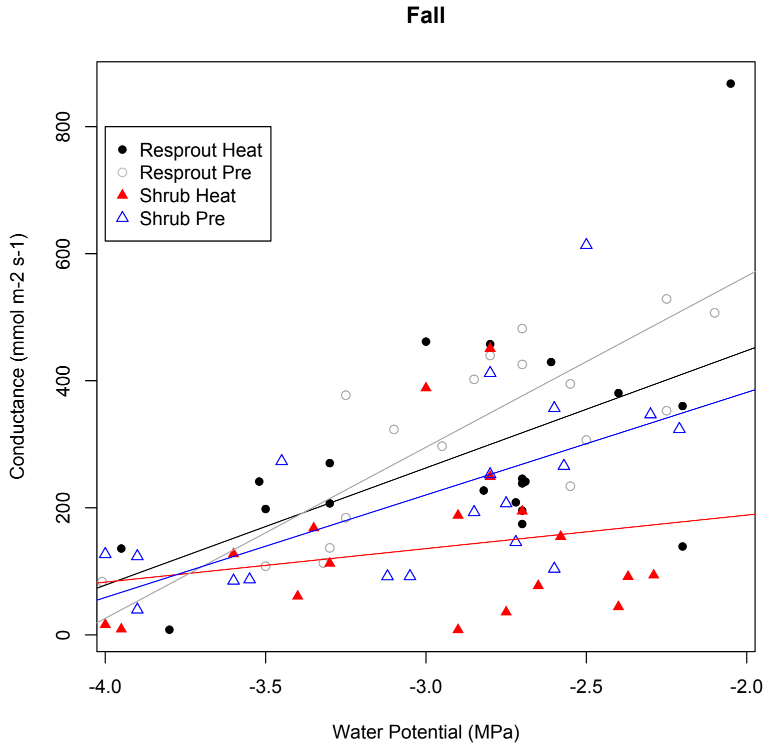

A
Resprouts
Pre
Heat
B
Shrubs
Electron Transport Rate (umol m^{-2} s^{-1})
120
100
80
60
40
20
0
*
0
2
4
6
8
10
Hours after sunrise

Figure 11





Supplemental Figure 1.

TABLES

Season	**Day**	**Date**	**g_s**	**Fv/Fm**	**ETR**	**Discrimination**	**Water Potential**
Spring	Pre	4/18/09	Yes	--	--	Yes	Yes
Spring	Heatwave	4/20/09	Yes	--	--	Yes	Yes
Fall	Pre	9/16/10	Yes	Yes	Yes	Yes	Yes
Fall	Pre	9/23/10	Yes	--	--	--	--
Fall	Heatwave	9/27/10	Yes	Yes	Yes	Yes	Yes
Fall	Heatwave	9/30/10	Yes	--	--	Yes	--

Table 1. Sampling matrix of measurements taken on different sampling dates. See methods for descriptions of methods. ETR = electron transport rate, g_s = stomatal conductance.

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352.

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CHAPTER 3

Seasonality affects the relative differences in ecophysiogy of mature and resprouting individuals of Heteromeles arbutifolia

Abstract

Previous research has shown that resprouting individuals have higher physiological rates following disturbance than mature individuals of the same species. However, most studies focus on comparisons immediately following a disturbance. We determined if differences persist 5-years post-disturbance by examining seasonal patterns of instantaneous photosynthetic rates, carbon isotope composition of leaf sugars, water-use, and chlorophyll fluorescence in resprouting individuals and mature individuals of *Heteromeles arbutifolia*. We found that many aspects of the physiology the resprouts had converged with those of mature individuals. Both types of plants had access to similar soil water reserves, similar water use efficiency, and experienced similar stress to photosystem II. However, resprouts had higher photosynthesis at the end of the dry season. This suggests that the differences between mature and resprouting shrubs are more pronounced when leaves are experiencing water limited conditions. If differences between shrubs and resprouts are more pronounced when leaf water potential and soil water availability are low, this could have important implications during drought years or under projected climate change if the precipitation regime changes. To assess the relative risks to an obligate resprouting species it is important to understand the long-term performance of both forms of the plant.

Introduction

Plant evolution and diversity have been influenced by fire (Keeley 2012, Pratt et al. 2012, Bond 2001) different life history types can be defined by their responses to crown fires (Keeley 2012, Pratt 2012). Obligate sprouters regenerate after a fire from shoots produced by belowground meristems fueled by non-structural carbohydrate reserves present in lignotubers (Keeley et al. 2012; Pratt et al. 2012; Paula and Ojeda 2009; Bell and Pate 1996; Kozlowski 1992; Bond and Midgley 2001). The resprouting strategy is found in various lineages from disturbance-prone environments (Keeley et al. 2012; Pratt et al. 2012; Keeley et al. 2011). In disturbance-prone environments resprouting has a stabilizing influence on populations because it confers persistence in the face of potentially catastrophic disturbances, such as crown fires (Lawes and Clarke 2011). There is strong evidence that the resprouting trait is adaptive (Keeley et al. 2011; Lamont and Downes 2011; Lamont et al. 2011) and that resprouting may also select for altered physiology compared to individuals that have not or cannot resprout (Hernández et al. 2011; Goorman et al. 2011; Paula and Ojeda 2009; Pratt et al. 2007; Bond and Midgley 2001; Fleck et al. 1998; Castell and Terradas 1994).

Immediately following a crown-destroying disturbance, resprouts mobilize carbon reserves to regrow. At some critical point they also must allocate an important fraction of their photosynthate to refill non-structural carbohydrate reserves so they can respond to subsequent disturbances. This is particularly important in the case of fire, which can have a very rapid return interval in some ecosystems (Goorman et al. 2011; Paula and Ojeda 2009). Resprouts need to fix, allocate and mobilize carbon stores, and resprouting individuals often have higher photosynthetic rates (as well as higher stomatal conductance and higher transpiration rates, Table 1) compared with mature plants (Paula and Ojeda 2009; Wildy et al. 2004; Fleck et al. 1998; Fleck et al. 1996; Bell and Pate 1996; Castell and Terradas 1994; Kozlowski 1992; Schlesinger and Gill 1980). Numerous studies have examined the physiology of resprouts in comparison to non-sprouting and intact mature shrubs or seedlings (Wildy et al. 2004; Fleck et al. 1996; Schlesinger and Gill 1980). Typically, these studies have either compared shrubs with regrown stems (referred to here as resprouts) to mature shrubs (referred to here as shrubs) or to seedlings following a recent disturbance. These studies have shown major physiological differences between young resprouts and mature shrubs, but little information exists on the physiology of older resprouts and the time frame of their physiological convergence with older stems.

In this study we asked if resprouts and mature shrubs of the widespread evergreen plant *Heteromeles arbutifolia* continue to respond differently to seasonal patterns in water availability and temperature many years after a disturbance. The resprouting individuals in our study had resprouted due to mechanical removal of their canopies four years prior to the onset of the study (other examples of non-fire resprouts include (Fleck et al. 1996; Shelden and Sinclair 2000)). This allowed us to study the physiology of resprouting without the potential confounding impacts of fire. We test the hypothesis that the carbon economy and water use between resprouts and mature shrubs will converge many years after a disturbance. Understanding the comparative physiology of resprouts and mature shrubs will be important for informed management and conservation as plants from Mediterranean-type climate regions are expected to be at high risk from fire-related disturbances under changing climates (Bond and Midgley 2001; Pratt et al. 2012). Species that have traits that are adaptive under a particular fire regime can become threatened when the fire regime changes (Keeley et al. 2011).

We measured a variety of physiological variables diurnally and seasonally, including leaf and stem water potentials (Ψ_{leaf} and Ψ_{stem}, respectively), leaf gas exchange, and chlorophyll fluorescence in mature shrubs and resprouts growing together over the course of two years. The resprouts at our site had a different architecture from the mature shrubs, with the resprouts having more individual stems and shorter stature (M. Shuldman). Due to their different growth forms we hypothesized that resprouting individuals had not yet converged on the same growth patterns as the mature shrubs and that at our site the resprouts would still exhibit the typical pattern of increased physiological activity when compared to mature shrubs. We expected resprouts to have higher photosynthetic rates, electron transport rates, transpiration, conductance, and leaf hydraulic conductance (K_{leaf}). Higher rates in these parameters would be consistent with increased physiological activity of resprouts as they grow rapidly and store carbon reserves post-disturbance.

Materials and Methods

Species description

H. arbutifolia is a widespread California native shrub that occurs primarily from Humboldt and Shasta counties in northern California to the San Pedro Martir Mountains in Baja California, but scattered populations extend as far south as La Paz in Baja California (Morrow and Mooney 1974). Unlike many chaparral shrubs, it has large, persistent leaves. *H. arbutifolia* is an obligate resprouter, with plants resprouting vigorously after fire, and seedlings only establish in the understory during fire-free intervals (McMurray 1990). Full-grown shrubs are typically 2-10m high (Munz 1973)and have an intermediate rooting depth (Ackerly 2004; Davis and Mooney 1986). While *H. arbutifolia* experiences substantial water deficit in midsummer (Ackerly 2004; Davis and Mooney 1986; Calkin and Pearcy 1984) it tolerates drought by maintaining leaf turgor and gas exchange in the dry season through shifts in osmotic potential and the bulk modulus of elasticity (Davis and Mooney 1986).

Site description and sampling design

Mediterranean-type climates are often characterized by high fire frequencies (Keeley et al. 2012). In these ecosystems, the demand for water is highest during the hottest and driest part of the year, as many evergreen plants grow and reproduce despite declining soil water potentials and increasing temperatures. The phenology of growth and reproduction are tightly coupled to seasonal cycles of temperature, light, and water availability(Cleland et al. 2007; Rathcke and Lacey 1985). In California's Mediterranean-climate ecosystems, leaf flush and flowering (Godoy et al. 2008) begin in winter or spring and often continue throughout the summer. The carbon-based energy required for growth and reproduction must be coupled with enough water to maintain transpiration and for cell expansion via turgor pressure.

Quail Ridge Natural Reserve (38°49'04" N, 122°14'28" W) located in Napa County, CA lies on a peninsula in the Berryessa Reservoir on the eastern edge of the Coast Range. It is made up of a patchwork of vegetation types, including grasslands, chemise chaparral, and oak woodlands. Elevation ranges from 34 to 462 m and the average yearly rainfall is 62 cm (Boucher et al. 2004). The topography is complex and consists entirely of soils derived from Lower Cretaceous-Upper Jurassic marine mudstone, siltstone, sandstone, and conglomerate. Our site was located on a ridge top at a chaparral-grassland ecotone, above a chemise chaparral site that burned in the fall of 2005. In 2005 some of the plants at our site had their canopies mechanically removed to form a firebreak. The site itself never burned in the 2005 fire. This left our site with interspersed intact, mature shrubs and resprouting shrubs. Climate data (Figures 1 and 2) were obtained from Markely Cove station, located less than 2 km from our site (Western Regional Climate Center, http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca5360).

We concentrated our sampling efforts in the spring and summer beginning in April 2009 and ending in November 2010. In 2009, we sampled the plants monthly from April-September and then in November after the first rain of the wet season. In 2010, we sampled monthly from April to November but also included an earlier wet season sampling period in February. During all sampling points we sampled the plants 4-5 times over a diurnal time course starting at before dawn and then sampling every 2-3.5 hours. The dates where plants were only sampled 4 times occurred at times of the year when day length was too short to allow for five sampling periods.

Leaf gas exchange and fluorescence

We measured stomatal conductance (g_s), transpiration (E), and leaf temperature (T_{leaf}) on two leaves of 5 resprouts and 5 shrubs at each time point using a LiCOR 1600 porometer (LiCOR Bioseceince, Lincoln, NE) on a monthly basis in 2010. In 2010 we also measured chlorophyll fluorescence and electron transport rate (ETR) on the same leaves that we measured stomatal conductance and water potential (WALZ Mini-Pam Heinz Walz GmbH, Effeltrich, Germany). In 2010 we sampled leaves at predawn to test for the potential quantum yield of photosystem II by measuring fluorescence with the light pulse on the fluorometer set to low and sampled leaves during the daytime to determine the ETR with the light pulse set to high. Whole plant conductance was estimated as:

$$(1)\ g_{plant} = \frac{E_{max}}{\Psi_{max} - \Psi_{min}}$$

where E is maximum transpiration, $\Psi_{max}$is the maximum leaf water potential and Ψ_{min} is the minimum leaf water potential.

At the end of the rainy season (May 2010) and the end of the dry season (October 2010) we measured gas exchange using a LiCOR 6400. We measured 4 resprouts and 4 shrubs at 4 time points throughout the day. The chamber temperature was allowed to fluctuate with ambient air temperature and CO_2 levels were fixed at 400 ppm. We stepped up the light levels from 500 μmol to 1500 μmol in 500 μmol increments, letting the plants equilibrate to each level before moving to the next. We took measurements at 1500 μmol. Before each measurement we took an empty chamber measurement and subtracted the empty chamber measurement to determine gas exchange rates.

Water Potential

During each sampling period we collected leaf and branch water potential using a Scholander-style pressure chamber (Plant Moisture Stress, Albany, OR). We sampled plants before sunrise and 3-4 other times throughout the day. Leaves were excised with a razor blade, immediately wrapped in plastic wrap, put in a small ziptop bag, and placed in a dark cooler until they were measured. Leaves remained in the cooler for less than 90 minutes. Data gathered at the University of California, Berkeley campus using the same method indicated that there was no significant difference in water potential for leaves on the same branch if they were measured immediately or after sitting wrapped and in the dark cooler for 90 minutes (data not shown). For the stem water potential measurement we wrapped leaves on the plant in plastic wrap, covered them with aluminum foil, and wrapped a small piece of Parafilm (Pechiney Plastic Packaging Company, Chicago, IL) at the petiole in the late afternoon prior to the day of the measurements. When we were ready to sample, we excised the leaf, put it in a small ziptop bag and placed it in a dark cooler. Every effort was made to select leaves for leaf and branch water potential from the same branch. Using transpiration from the LiCOR 1600 porometer and leaf and branch water potential we estimated leaf hydraulic conductivity (K_{leaf}) using the following equation:

$$(2)\ k_{leaf} = \frac{E}{\Psi_{leaf} - \Psi_{branch}}$$

where E is transpiration, Ψ_{leaf} is the leaf water potential and Ψ_{branch} is the branch water potential.

Carbon Isotope Discrimination

We sampled leaves from throughout the plant canopies at approximately 16:00 on each sampling day. Leaves were cut, placed in a manila coin envelope, immediately frozen in liquid nitrogen, and stored in a ziptop bag on ice until they were placed in a freezer. We then freeze dried the samples, removed the petioles, and ground the lamina into a fine powder. Using a modified protocol(Brugnoli et al. 1988) we removed the leaf sugars by extracting 150 mg of ground leaf material in a 1:1 aqueous solution of Polyvinylpolypyrrolidone (PVPP, Sigma-Aldrich, Inc., St. Louis, MO). We removed amino acids from the solution with an ion exchange resin (Dowex 50WX8-100, Sigma Aldrich Inc., St. Louis, MO) and organic acids with an anion exchange resin (Dowex 1X2, Sigma Aldrich, Inc., St. Louis, MO). The final extract was freeze dried and analyzed with an elemental analyzer (ANCA/SLPDZ, Sercon, Cheshire, UK) coupled to a continuous flow isotope ratio mass spectrometer (Finnigan MAT, DeltaAPlus XL, Thermo Scientific, Bremen, Germany). The carbon isotope ratios (‰) were expressed relative to the international standard V-PDB. The carbon isotope discrimination was calculated as:

$$(3)\ \Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}/1000}$$

where Δ is discrimination, $\delta^{13}C_{air}$ is the isotope ratio of the air and $\delta^{13}C_{leaf}$ is the isotope ratio of the leaf sugars. The $\delta^{13}C_{air}$ of the atmosphere was determined as the average value of the source air at the field site from three sampling times (4/2009, 5/2010, 11/2010). We sampled the source air by filling a 60 ml syringe with air and slowly expunging it into a 12ml Extetainer vial (Labco Limited, Buckinghamshire, England). We took quality control samples by sampling from a compressed air tank of know isotope value that was transported to the field. All quality control samples were treated like the unknown samples. We analyzed the samples within 48 hours of collection using a Thermo Gas Bench II coupled to a Thermo Delta Plus XL continuous flow isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). We calculated carbon isotope ratios of the air relative to the international standard V-PDB. There were no significant differences in $\delta^{13}C_{air}$ between the sampling dates (P=0.06, one way repeated measures ANOVA performed in Sigma Plot, Systat Software, Inc.) and therefore we used the average carbon isotope value in our calculation of discrimination (-8.59 ‰).

Statistics

Diurnal time courses and monthly data were analyzed using repeated measures MANOVA in JMP (SAS Institute, Cary, NC). If there were two levels of repeated measures (e.g. time of day and month) then a compound repeated measures MANOVA was used.

Results

Gas Exchange

We found that photosynthesis (*P* <0.0001), transpiration (*P* = 0.0007), and conductance (*P* <0.0001) were all significantly higher in resprouts at the end of the wet season (May 2010) than at the end of the dry season (October 2010, Figure 3). We sampled plants at four time points during the day, but in both seasons there was no significant difference between time of day (*P* = 0.24). Resprouts had higher photosynthesis than shrubs in October, but not in May (*P*=0.02).

Discrimination

There were no significant differences between shrubs and resprouts in carbon isotope discrimination assessed with leaf sugars (Figure 4, *P* = 0.18)). There were significant differences between sampling days (*P* = <0.0001) but no significant interaction between sampling day and plant type (*P* = 0.13).

Fluorescence

We found no significant difference between shrubs and resprouts in Fv/Fm measured before dawn (*P* = 0.87; Figure 5). Throughout 2010 both types of plants had predawn Fv/Fm values near 0.8, but this dropped dramatically below 0.8 in November 2010. There was also no significant difference between shrubs and resprouts for midday electron transport rate (*P* = 0.47; Figure 5).

Seasonal Water Potential

We measured plants 3-4 times during the day, depending on day length, and were able use these data to determine the maximum water potential (least negative) and minimum water potential (most negative). In general, there was no difference between shrubs and resprouts in minimum leaf water potential during either year (*P* = 0.49), although there was a significant interaction between type and day (*P* = 0.04). Post hoc analyses showed that shrubs had less negative water potentials than resprouts in April 2009 (Figure 6A and Figure 6B). There were differences between the different sampling dates (*P* <0.001), with water potential decreasing throughout the dry season. Maximum water potential did not differ between shrubs and resprouts in either year (*P* = 0.24; Figure 6C and Figure 6D). Although the maximum water potential declined throughout the dry season, the variability in maximum water potential increased throughout the dry season. Furthermore, there was a significant effect of sampling date on maximum water potential (*P* <0.001).

Seasonal physiology

For a given leaf temperature resprouts had a higher transpiration and conductance than shrubs (Figure 7), but they did not have higher hydraulic capacity (i.e. higher K_{leaf}) or a difference in leaf water potential. Stomatal conductance of both shrubs and resprouts declined with decreasing leaf water potential (Figure 8), however this decline occurred at less negative water potentials for shrubs (approximately -3 MPa), than for resprouts (approximately -3.5 MPa). Whole plant conductance varied with the day of the year (P = <0.001), but there were no differences between shrubs and resprouts (P = 0.14; Figure 9).

Discussion

We found some evidence that resprouting individuals maintain higher physiological activity 4-years and 5-years post-disturbance compared with undisturbed mature plants as evidenced by their higher photosynthetic rates, electron transport rates, and stomatal conductance. We did not find evidence that K_{leaf} was greater in resprouts. Some of our findings are consistent with increased physiological activity of resprouts as they grow rapidly and store carbon reserves post-disturbance, but our data indicate that 4-years post-disturbance resprouts and shrubs at this site are converging on similar physiology. When we estimated whole plant conductance we found that there were no differences between shrubs and resprouts. Since resprouting is a whole plant phenomena, this is further evidence that the plants are converging.

Several studies have shown that resprouting alters physiology compared to mature, non-resprouted shrubs (Hernández et al. 2011; Pratt et al. 2007; Castell and Terradas 1994; Fleck et al. 1998) . *H. arbutifolia* has belowground meristems found in a lignotuber. Like many plants that use a resprouting strategy, the meristems are initially fuelled by non-structural carbohydrate reserves but continued growth and refilling of starch reserves is dependent on high photosynthetic rates that often correlate with high stomatal conductance, and high transpiration rates as well (Wong et al. 1979). Another potential explanation for enhanced activity in resprouts is that they temporarily revert back towards a pre-reproductive or juvenile stage (Iwasa and Kubo 1997). Juvenile woody plants typically have higher rates of photosynthesis, stomatal conductance, and transpiration than adults (Goorman et al. 2011; Niinemets 2010; Thomas and Winner 2002; Bond 2000; Donovan and Ehleringer 1991); however, the resprouts in our study were reproductive. Despite the longer interval between disturbance and our sampling period compared to some previous studies, we found that resprouts still maintained higher photosynthetic rates in the fall of 2010 when soil water availability was low (Figure 3).

There were no significant differences between photosynthesis in shrubs and resprouts in the spring of 2010 when soil water was abundant. In addition, there was a trend for resprouts to have higher ETR, but the difference was not significant (Figure 5). Total precipitation in 2010 was 874.8mm, which is higher than the 29-year average of 732.8mm (Figure 2). In a dry year, such as 2009 (Figure 6), predawn water potential would likely be lower and differences between shrubs and resprouts may have been more pronounced as plant experienced lower leaf water potential, In general, we found no significant seasonal differences between shrubs and resprouts.

There was no significant effect of season on minimum and maximum water potential, carbon isotope discrimination, or Fv/Fm. This means that the two types of plants had access to similar soil water reserves, similar water use efficiency, and experienced similar stress to photosystem II. In a study that examined resprouting and un-burned *Quercus ilex* plants one year post-disturbance, photosynthesis and conductance were similar between the two plant types earlier in the dry season, but later in the dry season the resprouts showed higher photosynthetic rates and stomatal conductance (Fleck et al. 1998). Similar to our study they found no differences between the two plant types in Fv/Fm, suggesting that photoinhibition was not occurring(Fleck et al. 1998). When we pooled all the seasonal data to examine how the entire range of leaf temperature influenced transpiration, conductance and water potential, we found some evidence that resprouts had higher transpiration rates but not higher K_{leaf}.

Plants at our site experience a wide range of soil water potentials over the course of the year ranging from close to -1 MPa to close to -4.5 MPa (Figure 6). We hypothesized that resprouts would have higher transpiration and conductance, and in turn they would have higher hydraulic capacity. We found that for a given leaf temperature transpiration and stomatal conductance were higher in resprouts than in shrubs (Figure 7), but we did not find differences in K_{leaf} between resprouts and shrubs (Figure 7). In retrospect this should not be surprising because there is evidence that $\Delta\Psi_{leaf}$ converges during peak transpiration for plants of a given system, even if there are variation in plant size, age, rooting depth, or vulnerability to cavitation (Sack and Holbrook 2006; Brodribb et al. 2005; Nardini and Salleo 2000; Stratton et al. 2000).

Conclusions

If the differences between shrubs are resprouts are more pronounced when leaf water potential and soil water availability are low, then this could have important implications if global climate change results in changes to the precipitation regime. Our data show that 4-years and 5-years post disturbance shrubs and resprouts converge on many aspects of their physiology. However, resprouts had higher photosynthesis at the end of the dry season and higher conductance during times of when leaf water potentials were low (i.e. below -3MPa). This suggests that the differences between shrubs and resprouts are more pronounced when leaves are experiencing water limited conditions. Plants from Mediterranean-type climate regions are expected to be at high risk from global change (Bond and Midgley 2001; Parry et al.; Pratt et al. 2012a)and to determine the relative risks to an obligate resprouting species it is important to understand the long term performance of both forms of the plant.

Figures

Figure 1. Mean monthly air temperature and precipitation from Western Regional Climate Center's Markley Cove station. Data plotted are the National Climatic Data Center normals from 1981-2010.

Figure 2. Monthly precipitation (A) and average maximum air temperature (B) for 2009 (black circles) and 2010 (white circles) from Western Regional Climate Center's Markley Cove station. The 29-year average monthly precipitation (A) and maximum temperature (B) is also plotted in grey based off the National Climatic Data Center normals from 1981-2010. Total precipitation was 549.9mm in 2009 and 874.8mm in 2010. The 29-year average total precipitation was 732.8mm.

Figure 3. Photosynthesis, transpiration, and conductance measured over a diurnal time course at the end of the wet season (May 2010, A, C, E) and the end of the dry season (October 2010, B, D, F). Photosynthesis (P <0.0001), transpiration ($P = 0.0007$) and conductance (P <0.0001) were significantly higher in the wet season than the dry season. In October, resprouts had higher photosynthesis than shrubs ($P = 0.02$), but there was no significant different between shrubs and resprouts for the other variables measured. There were no significant effects of time of day.

Figure 4. We found no significant differences between shrubs and resprouts for monthly carbon isotope discrimination for 2009 and 2010 (n= 5 plants; $P = 0.18$). There were significant differences between sampling days (P = <0.0001).

Figure 5. In 2010 we found no significant differences between shrubs and resprouts for monthly measurements (shown in Julian day) of predawn Fv/Fm (A) or electron transport rate (µmol m^{-2} s^{-1}) at midday (B), $P = 0.87$ and $P = 0.47$ respectively.

Figure 6. The minimum and maximum daily leaf water potential for shrubs and resprouts during 2009 and 2010. We found that there were no significant differences between shrubs and resprouts for minimum water potential ($P = 0.49$) or maximum water potential ($P = 0.24$), except the shrubs had less negative minimum water potential that resprouts in April 2009 ($P = 0.04$).

Figure 7. Transpiration (A), conductance (B), leaf water potential (C), and leaf hydraulic conductance ((K_{leaf}; D) for shrubs (black circles) and resprouts (grey circles) plotted against leaf temperatures, all from 2010. Resprouts have higher transpiration and conductance for a given leaf temperature but there is no difference between shrubs and resprouts for leaf water potential of K_{leaf}.

Figure 8. Conductance (A) and transpiration (B) from monthly measurements throughout 2010 plotted against leaf water potential for shrubs (black circles) and resprouts (grey circles). There is a sudden decline in conductance and transpiration near -3 MPa for shrubs and -3.5 MPs for resprouts.

Figure 9. Whole plant conductance from monthly measurements throughout 2010 plotted against leaf water potential for shrubs (black circles) and resprouts (grey circles). Whole plant stomatal conductance varied with the day of the year (*P* = <0.001), but there were no differences between shrubs and resprouts (*P* = 0.14 ; Figure 9).

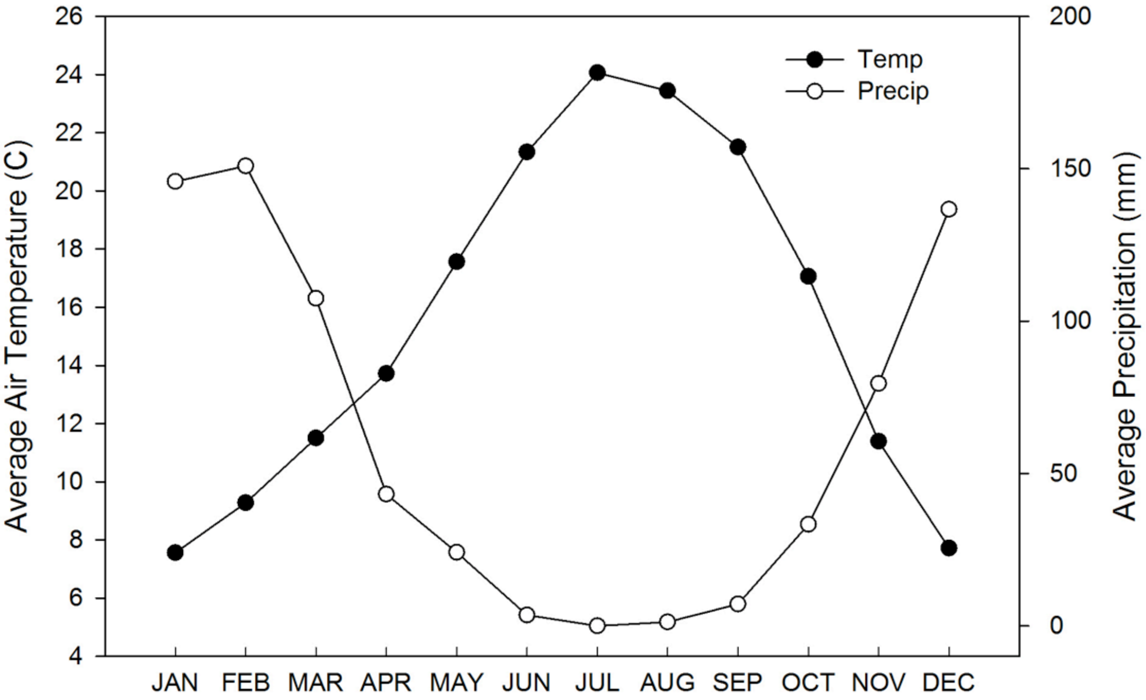
Average Air Temperature (C)
Average Precipitation (mm)
Temp
Precip
26
24
22
20
18
16
14
12
10
8
6
4
200
150
100
50
0
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Figure 1.

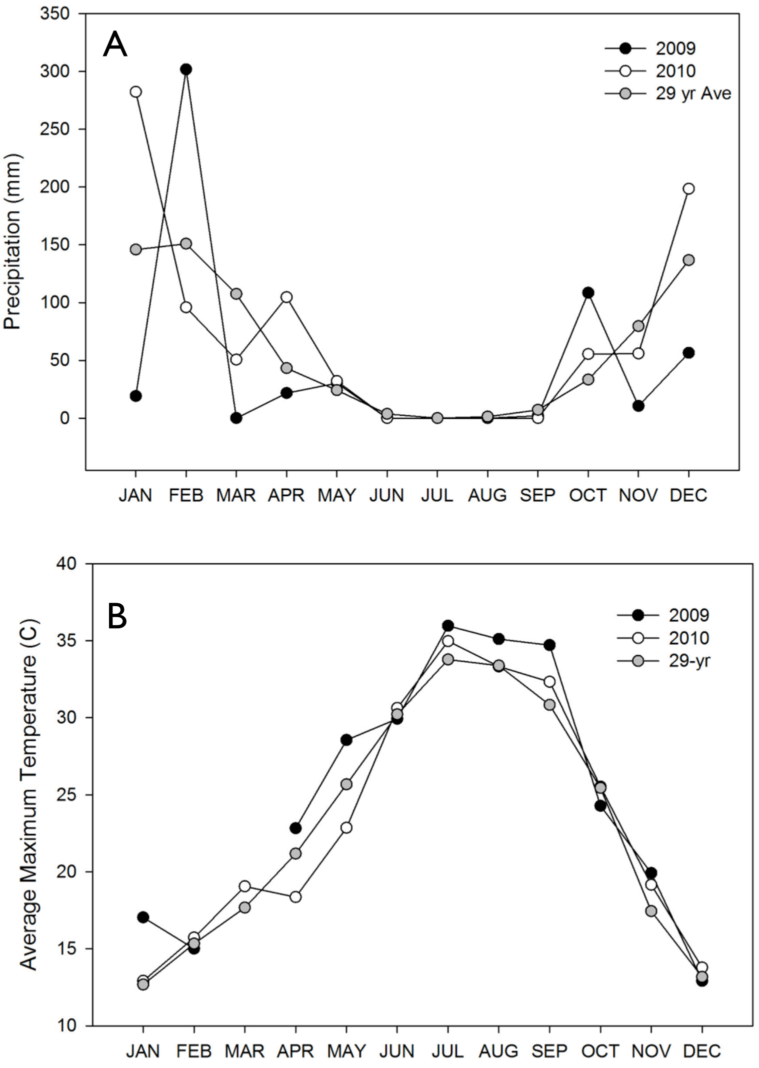
A
2009
2010
29 yr Ave
Precipitation (mm)
350
300
250
200
150
100
50
0
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
B
2009
2010
29-yr
Average Maximum Temperature (C)
40
35
30
25
20
15
10
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC

Figure 2.

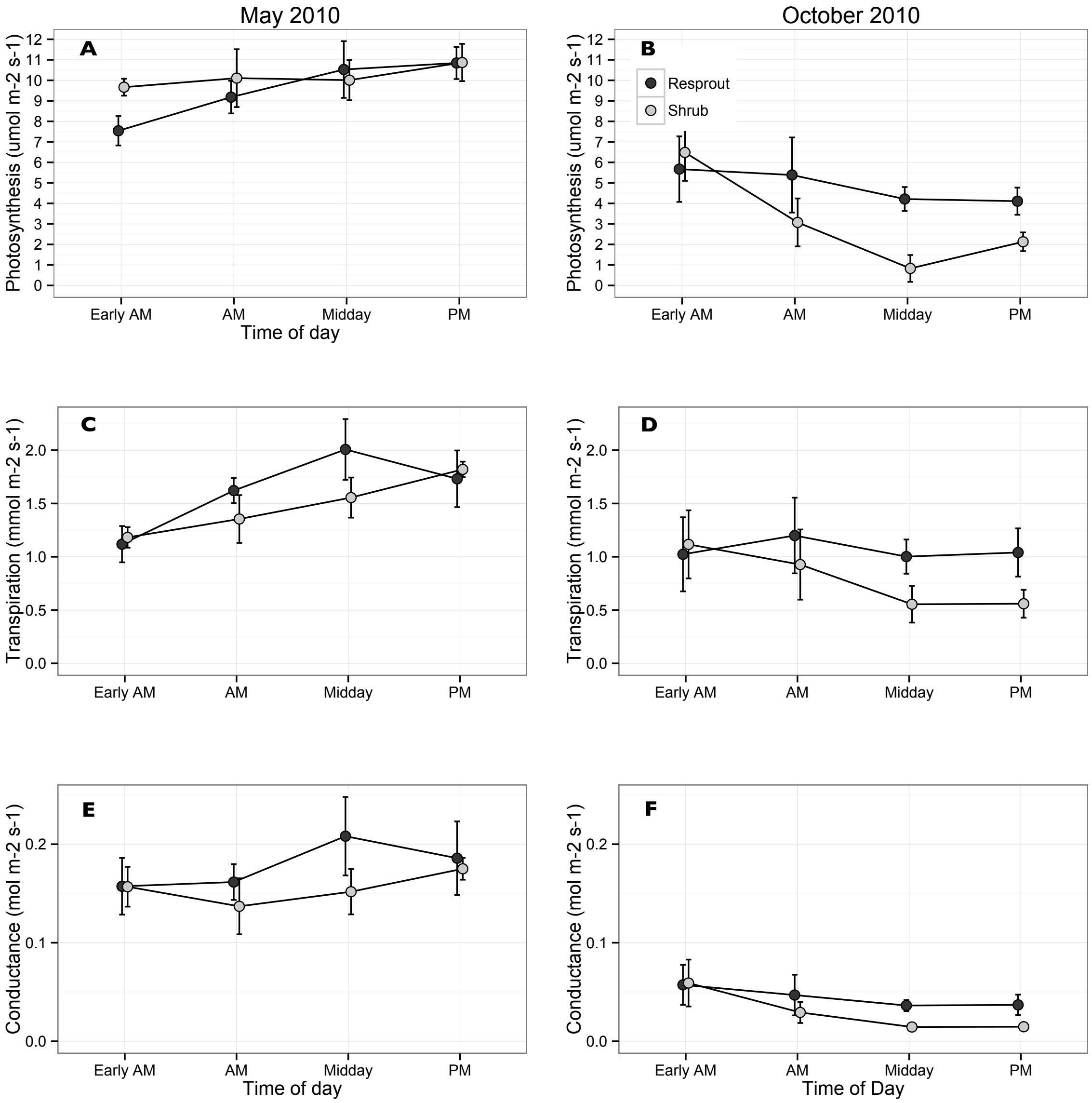
May 2010
October 2010
A
B
C
D
E
F
Resprout
Shrub
Photosynthesis (umol m-2 s-1)
Transpiration (mmol m-2 s-1)
Conductance (mol m-2 s-1)
0
1
2
3
4
5
6
7
8
9
10
11
12
0.0
0.5
1.0
1.5
2.0
0.0
0.1
0.2
Early AM
AM
Midday
PM
Time of day
Time of Day

Figure 3.

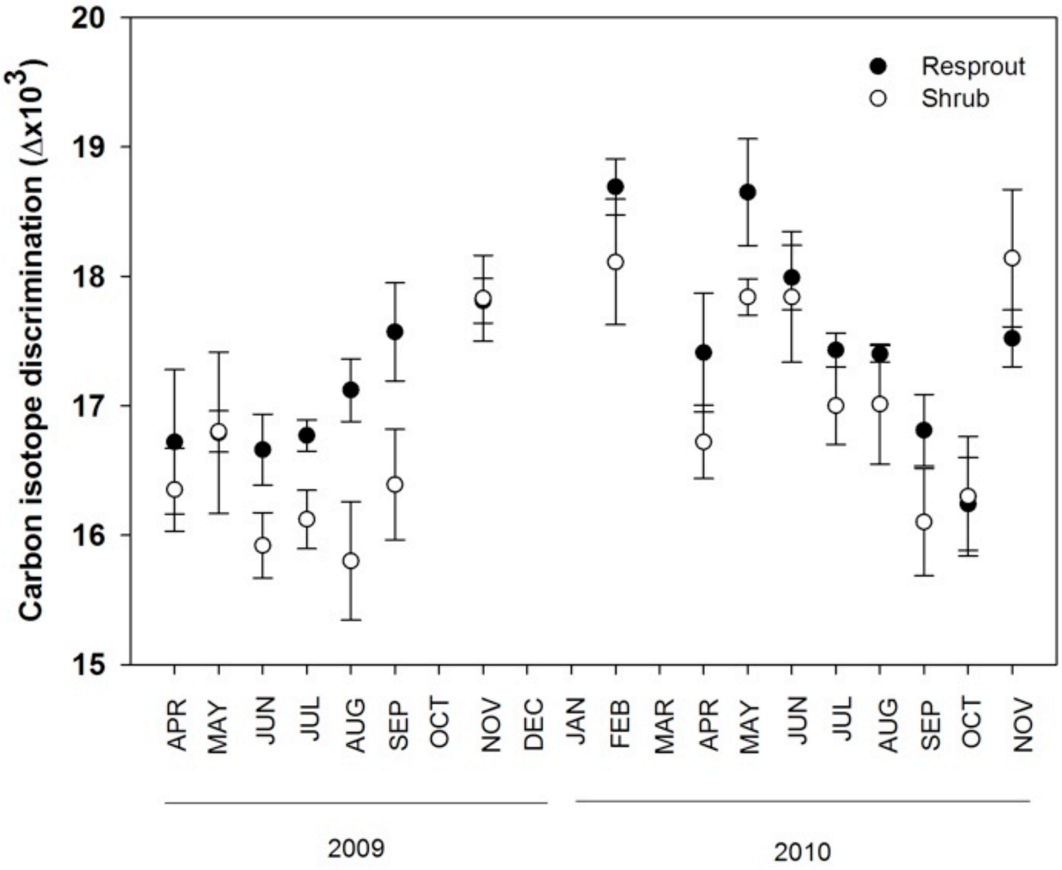
20
19
18
17
16
15
Carbon isotope discrimination (Δx10³)
● Resprout
○ Shrub
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
2009
2010

Figure 4.

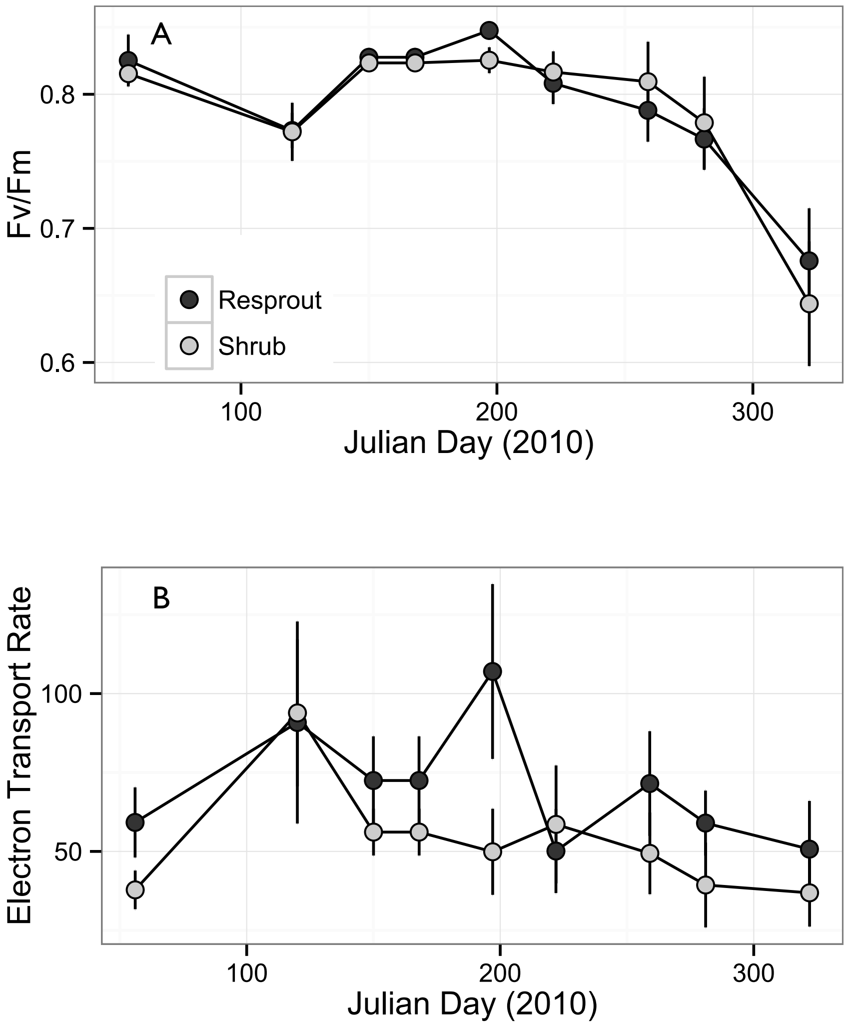
A
Fv/Fm
0.8
0.7
0.6
Resprout
Shrub
100
200
300
Julian Day (2010)
B
Electron Transport Rate
100
50
100
200
300
Julian Day (2010)

Figure 5.

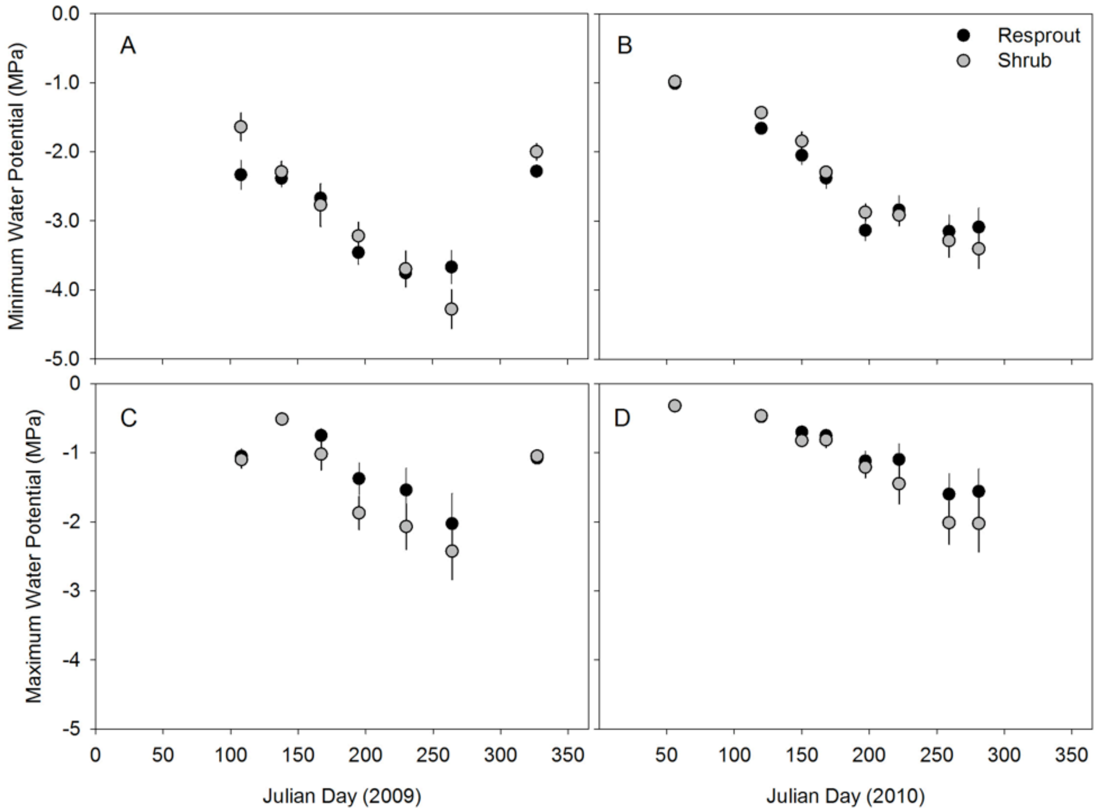
A
B
C
D
Resprout
Shrub
Minimum Water Potential (MPa)
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
Maximum Water Potential (MPa)
0
-1
-2
-3
-4
-5
0
50
100
150
200
250
300
350
Julian Day (2009)
Julian Day (2010)

Figure 6.

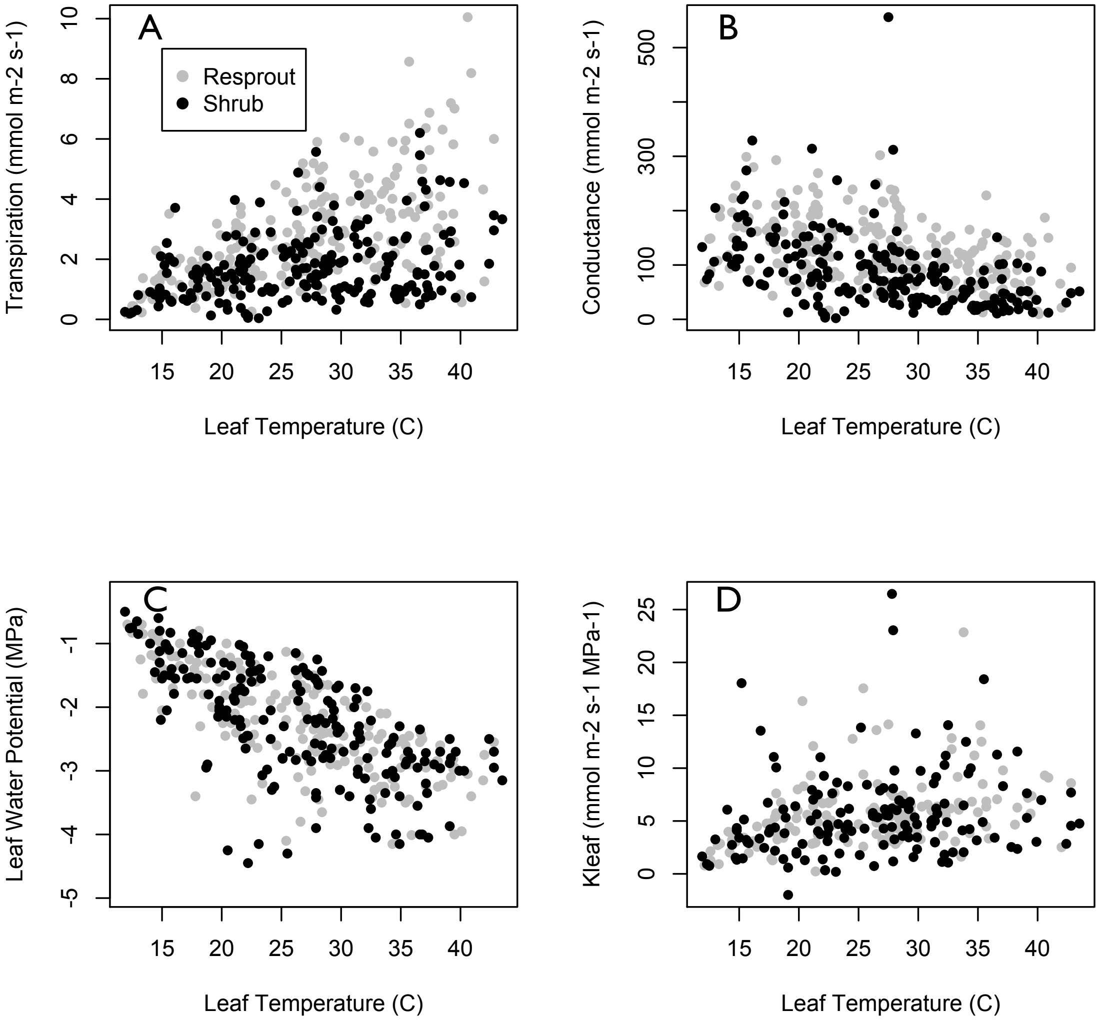
A
Resprout
Shrub
Transpiration (mmol m-2 s-1)
0
2
4
6
8
10
15
20
25
30
35
40
Leaf Temperature (C)
B
Conductance (mmol m-2 s-1)
0
100
300
500
15
20
25
30
35
40
Leaf Temperature (C)
C
Leaf Water Potential (MPa)
-1
-2
-3
-4
-5
15
20
25
30
35
40
Leaf Temperature (C)
D
Kleaf (mmol m-2 s-1 MPa-1)
0
5
10
15
20
25
15
20
25
30
35
40
Leaf Temperature (C)

Figure 7.

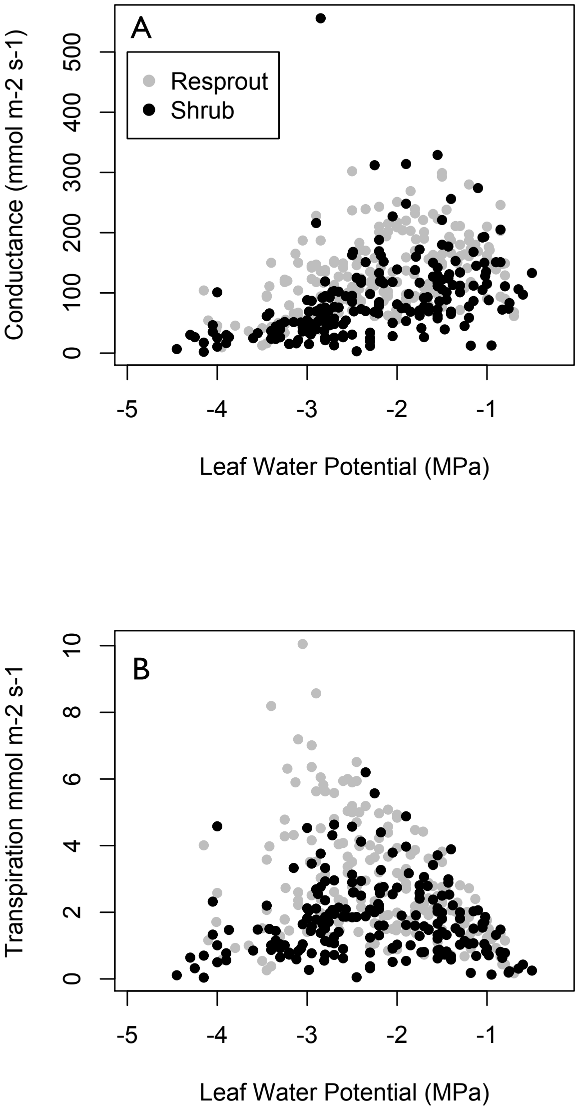
A
● Resprout
● Shrub
Conductance (mmol m-2 s-1)
0
100
200
300
400
500
-5
-4
-3
-2
-1
Leaf Water Potential (MPa)
B
Transpiration mmol m-2 s-1
0
2
4
6
8
10
-5
-4
-3
-2
-1
Leaf Water Potential (MPa)

Figure 8

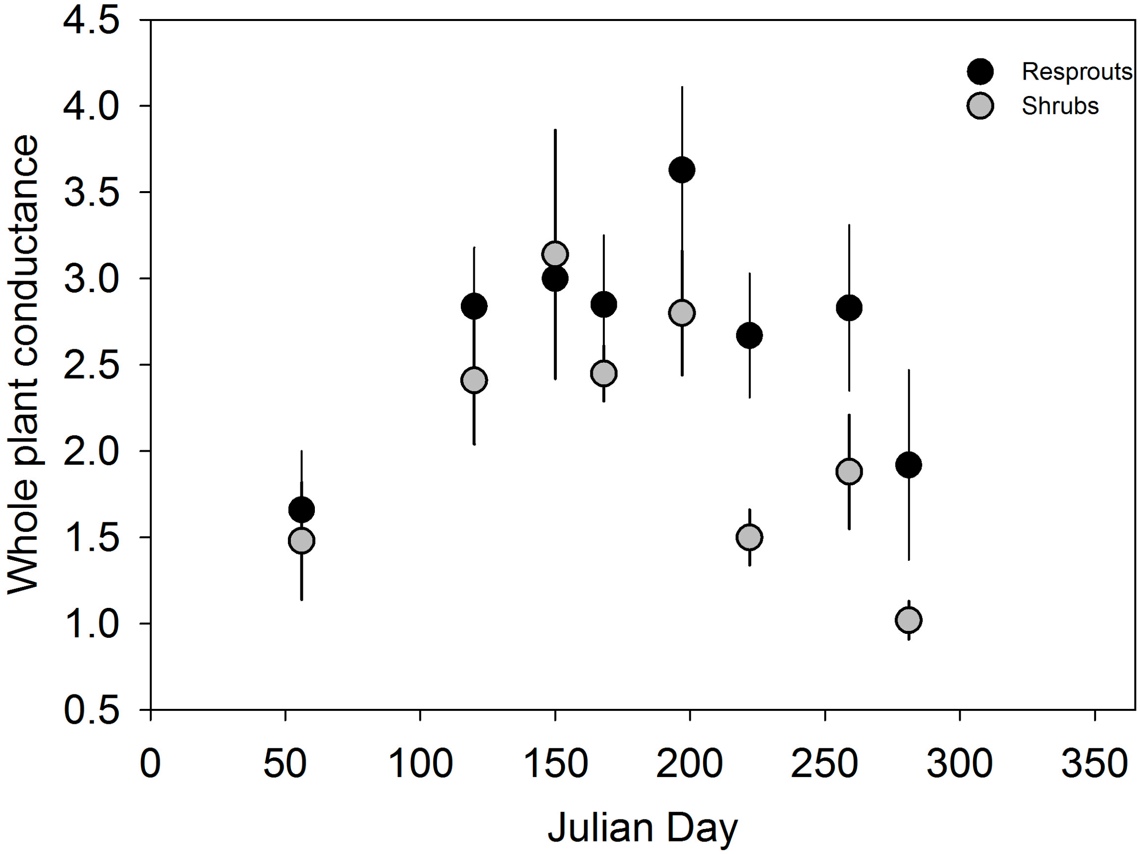
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Whole plant conductance
0
50
100
150
200
250
300
350
Julian Day
Resprouts
Shrubs

Figure 9

Study	Species	Growth form	Disturbance	Time since disturbance	Major Findings
Castell and Terradas 1994	*Arbuts unedo*	Shrub or small tree	Cutting	<1 year	Resprouts had higher Ψ, g_s, E, A, and RGR
Fleck et al. 1996	*Quercus ilex*	Tree	Cutting, fire	1 year	No differences between resprouts from cutting vs. fire; Resprouts had higher A, g_s, E and WUE
Bell and Pate 1996	*Leucopogon verticillatus; Conostephium pendulum*	Shrub	Fire	1-8 years	Tree ring analyses showed that resprouts grew fast immediately post-fire
Fleck et al. 1998	*Quercus ilex*	Tree	Fire	1 year	A and g_s higher in resprouts later in the dry season; Resprouts did not increase photoprotective compounds
Wildy et al. 2004	*Eucalyptus kochii*	Tree	Cutting	1-2 years	Resprouts had higher g_s, A and dry matter production but lower WUE
Paula and Ojeda 2009	*Erica spp.*	Shrub	Cutting	< 1 year – 2 years	Root starch levels lower in resprouts
Gorman et al. 2011	*Erica scoparia, E. australis*	Shrub	Cutting	< 1 year	Resprouts had higher A, light use efficiency, g_s, lower WUE

Table 1. Summary of results from the literature showing that it is typical for resprouts to have higher physiological rates than mature shrubs. A = photosynthesis, E = Transpiration, g_s = stomatal; conductance, Ψ = water potential, WUE = water use efficiency, and RGR = relative growth rate.

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