Plant Responses to the Urban Climate:
A Look at Stomatal Numbers and Growth of Plants
in Seattle, Washington

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This is to certify that I have examined this copy of a master's thesis by

Nicole Hackman

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

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1. Introduction

As the world's population approaches seven billion, the majority of people are living in cities and urban environments. Recent research suggests that 75-80% of North America's population is considered urban (Pataki *et al.*, 2006). As discussed in Onozaki (2009), there are links between the observed increase in global CO_2 concentrations, air temperature and the rise of the world's population. The increase in global atmospheric CO_2 concentration is directly attributed to anthropogenic sources of CO_2 primarily via the burning of fossil fuels for transportation, energy demands, and industrial manufacturing (IPCC, 2007). These facts culminate to reveal the concept that a rise in population, the size and number of densely populated urban areas or cities, fossil fuel combustion, and atmospheric CO_2 concentrations are linked. Further investigation of these connections has led researchers to look closer at the environmental variables of urban centers.

The term "Urban CO_2 Dome" refers to the relatively high concentration of CO_2 observed in the city of Phoenix, AZ., compared to surrounding locations (Idso *et al.*, 1998). It was found that cold season CO_2 concentrations averaged around 67 percent higher in downtown Phoenix than outside of the city where population and fossil fuel emissions are diminished. Further study revealed that peak vehicle traffic hours correspond with the highest CO_2 concentrations recorded, directly linking the elevated CO_2 levels to anthropogenic sources (Idso *et al.*, 2002). Trends similar to the urban CO_2

dome in Phoenix have been documented in several cities such as, Baltimore,

MD., and Vancouver, Canada (George *et al.,* 2007). In addition to the

elevated levels of CO_2, many cities have documented higher temperatures

compared to surrounding areas. This condition is known as the urban heat

island effect (George *et al.,* 2007; Oke and Maxwell, 1975). The direct effects

of human induced changes in CO_2 and temperature of urban locations make

for interesting inquiries into the indirect effects of these variables on urban

vegetation. However, the scope of such research is limited because

consistent records of the CO_2 levels in urban areas have not been available

until recently.

In April, 2009, the United States Environmental Protection Agency

(EPA) added CO_2 as well as five other heat trapping gases to a list of

pollutants that "endanger the public's health and welfare"(EPA, 2009). There

are many reasons why sampling CO_2 is necessary and given the EPA's recent

assessment it is likely that there will be an increasing need for the active

monitoring and sampling of CO_2 in cities throughout the United States. By

collecting CO_2 data, policy makers can make informed decisions about setting

realistic goals in order to decrease levels of the heat-trapping gas. In addition,

CO_2 data can be added to models that can help predict future trends in

atmospheric CO_2 patterns and other climate related scenarios. Furthermore,

every city is different in terms of its layout, infrastructure, traffic patterns and

geographic location, etc., and therefore may exhibit varying trends in CO_2

concentrations (Reid and Steyn, 1997). Active sampling of CO_2 is necessary because, while it may seem intuitive that environmental variables such as CO_2 concentrations and air temperature of urban areas are different than the surrounding locations, the degree and extent to which they differ is uncertain without the data as evidence.

At several urban sites where CO_2 and temperature have been monitored and documented, levels are comparable to the modeled predictions of future (50-100 years) global climate scenarios (George *et al.,* 2007). Monitoring these conditions and how plants are responding is a logical approach to understanding plant responses to climate change. Previous studies have documented these variations in environmental conditions across urban to rural transects and measured corresponding plant responses (e.g. George *et al.,* 2007; Gregg *et al.*, 2003; Ziska *et al.*, 2003). These approaches can lead to a greater understanding of the broader implications of climate change on vegetation and ecosystems.

Small-scale (e.g. cell to leaf level) physiological changes in plants such as altered stomatal density (number of stomata per unit area) can have large implications for the overall canopy conductance at regional and global scales such as altering the pattern of the hydrologic cycle at these scales (Field *et al.*, 1995). Woodward (1987) found evidence of a 40 percent reduction in the stomatal density of herbarium leaf samples from the last two centuries due to anthropogenic increases in CO_2 levels that ranged from 280 to 340 ppm.

Such a reduction in stomatal density can have further effects on leaf conductance, including a decrease in stomatal aperture (relative openness) and conductance (rate of being open) when exposed to elevated levels of CO_2 (Field, *et al.*, 1995). When the effects of these small-scale changes at the leaf level are measured at larger scales, the indirect effects of increased CO_2 levels from anthropogenic sources on an overall canopy conductance can be revealed. For example, in addition to the reduction in stomatal density as CO_2 increased from 280 to 340 ppm, there was a significant increase in water use efficiency (WUE) which includes photosynthesis and transpiration measurements (Woodward, 1987).

On a global scale, stomatal pores are responsible for a majority of the flux between the Earth's water and carbon cycles by means of respiration and transpiration as a result of photosynthesis (Hetherington and Woodward, 2003). Over large areas these effects can lead to an altered climate regime with increases in temperature and decreases in precipitation (Field, *et al.*, 1995). Other studies suggest similar connections between the effects of increased global CO_2 concentrations and decreased plant transpiration rates. As plants are losing less water through the process of evapo-transpiration they are using less water from the soil, thus their overall water use efficiency (WUE) rates are projected to increase (Gedney *et al.*, 2006). Researchers speculate these changes in plant physiology at small scales can lead to large-scale effects in the form of increased continental river runoff (Betts *et al.*,

2007). When the eco-physiological changes observed in cellular-scale response studies are broadened to whole-plant, canopy and ecosystem levels their combined effects on processes such as transpiration and gaseous exchanges with the atmosphere can be of great magnitudes.

Changes in the environmental conditions of urban centers, including CO_2 and temperature, can influence the biomass accumulation rates of different plant species. In Baltimore, MD., researchers found that CO_2 and nighttime temperatures are significantly higher than in surrounding rural areas, and that plants growing in the city exhibited significantly higher above-ground biomass and heights than the same species in rural locations (Ziska *et al*., 2004). Similarly, in New York City, urban effects on vegetation were documented when *Populus deltoides* plants growing in the city exhibited two times the biomass of those grown in surrounding rural locations (Gregg *et al.,* 2003).

The ability to discern the many ways plants respond to elevated CO_2 and temperature in urban settings today is necessary and relevant and will serve as a valuable resource to understanding the human impact on natural settings outside the urban environment. In this study the attempt to monitor urban climatic variables and detect whether plants are responding is two-fold. First, by monitoring CO_2 concentration and other environmental variables it can be determined if the city of Seattle has significantly different averages in CO_2 and temperature than the rural town of Forks, WA. Weather stations

equipped with CO_2, temperature, relative humidity and light sensors collected data during the same time period in Seattle and Forks throughout the summer and fall of 2008. Second, this research is designed to determine if the differences in CO_2 concentration are enough to induce physiological changes in plants growing at these sites. Such physiological changes were measured by examining whether there are significant differences in the stomatal numbers (density and index), biomass and the leaf area of two plant species growing simultaneously in sites in Seattle and Forks, WA. At the sites, two species of plants were grown and used as "phytometers"- a term referring to the use of plants as a measurement of changes in environmental conditions (Gregg *et al.*, 2003). *Arabidopsis thaliana* was chosen because it is a model research species with particular ecotypes known to respond to CO_2 by altering their stomatal numbers (Lake and Woodward, 2008). The cottonwood hybrid *Populus trichocarpa* X *Populus deltoides* was chosen for its rapid growth and known stomatal response to elevated levels of CO_2 (Gregg *et al.*, 2003; Miyazawa *et al.*, 2006).

In addition to the field portion of this research, a controlled experiment was conducted using chambers to expose plants to elevated and ambient CO_2 levels. The purpose of the chamber portion was to determine if the stomatal responses detected from plants grown in the controlled environments coincide with responses detected from plants grown in the field. Ultimately, it could then be determined whether CO_2 was the leading cause of change in stomatal

numbers. In the controlled environment setting both *Populus trichocarpa* X *Populus deltoides* and *Arabidopsis thaliana* (ecotypes Col-0 and WS) have been found to respond to elevated levels of CO_2 (approximately 700 ppm) by decreasing their stomatal density (Lake *et al.*, 2001; Woodward *et al.*, 2002; Miyazawa *et al.*, 2006). Previous studies have not documented the effects of elevated CO_2 concentrations on plant stomatal numbers using the urban environment as a field setting.

2. Materials and Methods

2.1 Site Description

Four locations within the city of Seattle (47°36′N 122°19′W) were chosen for the purpose of establishing weather data that was of interest to this experiment, in particular CO_2 concentrations. The locations are: the Seattle Department of Transportation (SDOT), the University of Washington Alumni Association (UWAA), the Washington Park Arboretum (WPA) and the Center for Urban Horticulture (CUH). These abbreviations (SDOT, UWAA, WPA, and CUH) will be used for the rest of this document.

The SDOT and UWAA sites were chosen because of their close proximity to areas with high vehicle traffic volumes. The SDOT site located at the corners of South Dearborn Street and 9th Avenue South in Seattle's International District, borders Interstate 5 with traffic flows estimated around 60,000 vehicles per 24 hours. The UWAA site is in the University District of

Seattle at the corner of 15th Avenue NE and NE 45th Street with 10,000-

15,000 vehicles per 24 hour period (SDOT Traffic Flow Maps, 2006).

The other two sites are located in less dense areas of Seattle. The

Washington Park Arboretum (WPA) is a 230 acre botanical garden and park

located in the Montlake neighborhood of Seattle. The Center for Urban

Horticulture (CUH) is in the Laurelhurst neighborhood adjacent to the 74 acre

Union Bay Natural Area.

The Olympic Natural Resources Center served as the rural or "control"

site for this experiment. Approximately 140 miles west of Seattle on the

Olympic Peninsula in Forks, WA (47°57′N 124°23′W), it is far from urban

areas. This site will be referred to as "Forks" for the remainder of this

document. All locations are close to sea level, between 150 and 300 feet.
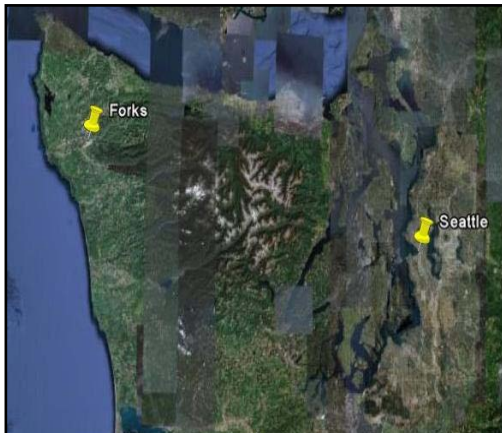
Fig. 1: View of northwest Washington state including the Seattle and Forks sites. (Google Earth v.5)
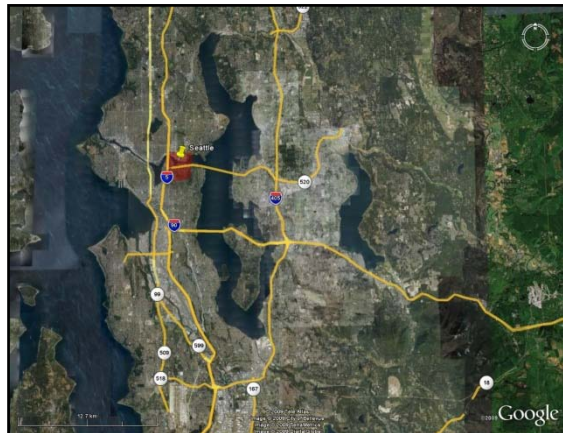
Fig. 2: View of Seattle and its' major highways. The red square indicates where the four sites within Seattle were concentrated. (Google Earth v.5)

2.2 Site weather monitoring stations

Two identical weather stations were constructed and equipped with sensors to monitor the following climatic variables: atmospheric CO_2 concentration (GMP343, Vaisala, Finland), relative humidity and air temperature (CS500, Campbell Scientific, Logan, Utah, USA). Two forms of light were also measured using a quantum sensor and precision pyranometer (SQ-100, and SP-110, Apogee Instruments, Logan, Utah, USA). Air samples were taken by the sensors at 30-second intervals, averaged every 15 minutes and stored in a CR10 programmable data logger/ controller (Campbell Scientific, Logan, Utah, USA). The sensors and the data logger were both powered using a 12-volt battery that was re-charged with a 10-watt solar panel (SP-10 Campbell Scientific, Logan, Utah, USA).

Prior to deployment, all sensors were factory calibrated and mounted onto portable, two meter tall instrumentation tripods (CM6 Campbell Scientific, Logan, Utah, USA). From July –October, 2008 both weather stations simultaneously monitored environmental variables between sites; while one station remained in Forks the other was deployed to one of the sites in Seattle at different time periods. The weather station was not used at the SDOT site due to security issues. Data were collected from the weather stations approximately every two weeks using the software PC200W 3.3 (Campbell Scientific, Logan, Utah, USA) and plotted using the statistical software package "R" (R Development core team, 2008).

2.3 Percent Canopy Openness

At each site hemispherical photographs were taken with a Nikon Coolpix 4500 Digital Camera and a Nikon FC-E8 Fisheye lens (Nikon Instruments Inc., Melville, New York, USA). Photos were acquired by mounting the camera onto a level (one meter high) tripod that was north facing and setting it to fisheye mode (Richardson, 2008). A photograph from each site was loaded into the program Gap Light Analyzer V. 2.0 (Simon Fraser University, Burnabay, B.C., Canada). From these photos the software extracts gap light transmission indices including percent openness.

2.4 Plant Materials

At all five site locations two species of potted plants were grown, the cottonwood hybrid *Populus deltoides x trichocarpa* (clone H-11-11) and *Arabidopsis thaliana* (Col-0 and Ws ecotypes). Both species were chosen for their likelihood to respond to elevated levels of CO_2 physiologically by altering their stomatal numbers (Lake *et al.*, 2001; Miyazawa *et al.*, 2005).

On May 22nd, 2008 *Populus deltoides x trichocarpa* (referred to as hybrid poplar for the rest of this document) cuttings were taken from the upper portion of a hybrid poplar tree that was planted in 1995 near Fife, WA. The cuttings were placed in a flat tray filled with a fine germination soil and grown under a misting bench for three weeks. Twenty-two cuttings rooted and each was planted into a two-gallon pot that was filled with well-watered, coarse

potting soil, and 20 grams of Osmocote® 13-13-13 slow-release fertilizer (The Scotts Company) mixed into the top layer. In July 2008, five poplar hybrids were moved to SDOT, WPA, and CUH while six plants were moved to UWAA and Forks. At each site a 20-gallon wading pool was used to submerge the bottom .05 meters of plants to assist in keeping plants consistently watered. Pots were wrapped in aluminum foil to reduce soil heating. Once at each site, the youngest two leaves of each plant were gently tagged to note the point at which new growth had been exposed to site conditions.

After seven weeks of growth the hybrid poplar plants were retrieved from all sites and brought to the laboratory for harvesting and analysis. Growth measurements were taken on every plant and include: height, diameter, total number of leaves, number of new leaves since tagging, and the number of lateral leaves. Four plants from each site were randomly selected for destructive harvesting so that the remaining plants could be used for future data collection. The total leaf area of each plant as well as each individual main stem leaf was obtained using a LI 3100c Area Meter (LI-COR, Lincoln, Nebraska, USA). Root balls of these plants were removed from the pots and washed to remove excess soil. All biomass except leaves from the main stem were then placed in a drying oven at 70°C for seven days and weighed to obtain the dry weight.

Main stem leaves from each pot were originally stored flat between plant presses in ascending order from their position on the plant, bottom to

top. Leaf impressions of both the adaxial (top) and abaxial (bottom) surfaces
were made by applying a dime-size amount of clear nail varnish half way
between the midrib and the edge of the leaf being sure to avoid main leaf
veins. Once the varnish was dry, clear tape was used to peel off the varnish
and seal it onto a glass slide. The slides for each plant were then examined
using a Nikon Eclipse E200 40x microscope (Nikon Instruments Inc., Melville,
New York, USA) and Nikon Coolpix 4500 Digital Camera (Nikon Instruments
Inc., Melville, New York, USA). Three images of each impression were taken
and imported into the image analyzing software Image J (Abramoff *et al.,*
2004). This software was used to calculate the area of the image and to keep
track of stomatal pore and epidermal cell counts for each image. Stomatal
density (number of stomata pores per unit area, mm^{-2}), epidermal density
(number of epidermal cells per unit area, mm^{-2}) and stomatal index (ratio of
stomatal pores to epidermal cells) were calculated using the numbers
produced by the Image J software. After impressions were made the main
stem leaves were placed in the drying oven at 70°C for seven days, then
weighed and added to the biomass calculation for each plant, giving total
above ground biomass measurements for each harvested hybrid poplar.

Arabidopsis thaliana (ecotypes: Col-0 and Ws) seeds were obtained
from The Arabidopsis Biological Resource Center (TAIR, Columbus, Ohio,
USA). In August 2008, one hundred seeds from each ecotype were placed in
a Petri dish and cold stratified in a refrigerator at 4°C for three days. For the

following two days, the seeds were placed in the greenhouse which was approximately 20°C where they began to germinate. Once two cotyledons formed, the seedlings were placed into half-gallon pots filled with a moistened fine peat soil that was amended with five grams of Osmocote® 13-13-13 slow-release fertilizer. These pots were wrapped with aluminum foil and two pots of each ecotype were then distributed out to the five sites. The plants began to flower after two to three weeks at which point they were collected from sites and brought to the lab where flower counts and impressions of the rosette leaves were made. All plant material was dried at 70°C for seven days, and weighed to obtain above ground biomass data. The entire process was repeated in September 2008.

The *A. thaliana* rosette leaves are small and fleshy compared to the hybrid poplar leaves and therefore required an additional step in making leaf impressions. Using the technique described in Lake *et al.* (2008), dental putty (Coltene Whaledent, Altstatten, Switzerland) was applied to both adaxial and abaxial surfaces of each rosette leaf to obtain a high-precision impression. With these permanent impressions of the leaf, nail varnish could then be applied and mounted onto slides as described for the poplar hybrid. Stomatal density, epidermal density and stomatal index were then calculated using the same techniques as described above.

2.5 Chamber experiment

The chamber experiment took place in the Douglas Greenhouse at the University of Washington Botanic Gardens from January 25th- March 3rd, 2009. Four closed-system chamber structures that have dimensions of 100 cm x 100 cm x 200 cm tall, with clear Mylar® walls were used to grow an additional set of the *A. thaliana* ecotypes (Kinmonth-Schultz., 2009). Two of the chambers were randomly chosen to serve as the elevated CO_2 treatment while the other two chambers received air from the greenhouse with levels of CO_2 considered ambient in this experiment. The two elevated chambers were connected to a 50-pound CO_2 tank with a known concentration of 700 ppm (Praxair, Seattle, WA., USA). The air passed through a series of tubes and a constant airflow was maintained with an inline fan and bubble flow meters (FL-2000, Omega, Stanford, CT., USA). Air samples of the chambers were made and recorded every 30-minutes with an infrared gas analyzer (CIRAS, PP Systems International, Inc., Amesbury, MA., USA) in order to monitor the diurnal CO_2 concentrations. The CIRAS infrared gas analyzer was calibrated prior to the start of the experiment at 0 and 700 ppm of CO_2. Air temperatures within the chambers were recorded at 15-minute intervals using a pair of thermocouples mounted at 20 and 100 cm from the top of each chamber and connected to a data logger (CR 1000, Campbell Scientific, Logan, UT., USA). Ambient light levels outside of the greenhouse were collected every 15 minutes using a greenhouse monitoring system (HortiMax, Rancho Santa Margarita, CA.,

USA).   Supplemental light is provided in the greenhouse from 8:00 am to 10:00 pm with high-pressure sodium 400-watt bulbs (Philips Electronics, Andover, MA, USA).

Four *A. thaliana* plants from each ecotype (Col-0 and Ws) were grown in individual pots for a total of eight plants per chamber. The seeds were placed in a Petri dish and cold stratified in a refrigerator at 4°C for three days. Seeds were then placed on top of pots filled with a fine peat soil that were generously watered to avoid dessication and had five grams of Osmocote[®] 13-13-13 slow-release fertilizer mixed into the top layer. The pots were placed in the chambers on small circular trays and watered regularly from the bottom as they had been in the field.   Seeds germinated in two days and began to flower in two to three weeks at which point the plants were removed from chambers and sampled. Sampling procedures for these plants consisted of counting the number of flowers, number of rosette leaves per plant and measuring total leaf area per plant using the LI 3100c Area Meter (LI-COR, Lincoln, Nebraska, USA). Leaf impressions of fully expanded rosette leaves were made using the same techniques described for the *A. thaliana* plants grown in the field. Finally, all above ground plant material was placed in separate paper bags, dried at 70°C for seven days, and weighed to obtain above ground biomass data.

2.6 Statistical Analysis

Weather station data was averaged over a 24-hour period for the duration the sensors remained at each site. Standard error for these values was calculated to show the variation in these points.

A general linear mixed model (R Development core team, 2008) was used to compare the plant response variables: stomatal numbers, epidermal density, biomass, flower numbers and leaf area between the fixed variables, sites (Forks, CUH, etc.) and chambers (elevated and ambient). An error term was written into the model to account for random variability between plants for the hybrid poplar. For the *A. thaliana* plants grown in the field, the error term accounted for variability between the two sets of plants as well as variability between individual plants. Pair-wise comparisons that were made between Forks and every other site (CUH, etc.) provided t-values showing the direction of change (increase or decrease) compared to Forks. Corresponding *p*-values from a *t*-distribution table (Zar, 1999) were obtained to determine the level of significance in the differences found between sites. The *p*-values are based on a two-tailed distribution and degrees of freedom are based on the number of pots at each site.

3. Results

3.1 Environmental Variables

Both weather stations were simultaneously recording data at two of the four sites (UWAA, WPA, CUH and Forks) we monitored. This allowed for comparisons to be made between the environmental variables of urban and rural locations from the same time period. The diurnal pattern of CO_2 represents the strong effect plants have on average CO_2 concentrations by means of photosynthesis. Typically, the maximum peaks in average CO_2 concentration are observed at night and early morning hours due to respiration and minimum concentrations are observed during the day due to the effects of photosynthesis. Data from this experiment reveals these diurnal patterns and the most significant differences in average CO_2 concentration are seen between the rural location of Forks and the CUH in Seattle (Fig. 3). The highest average CO_2 concentrations found at the CUH was 427ppm at the 700 hour (7:00 am) while 385 ppm was the maximum average CO_2 concentration reached at Forks, also at 7:00am. During mid-afternoon at both sites the lowest average CO_2 concentrations were recorded. At Forks the minimum average CO_2 concentration was 355 ppm at 1345 hours (1:45 pm) while the minimum average CO_2 concentration at the CUH, 383 ppm, was reached a few hours later at 1615 hours (4:15 pm). The trend of elevated CO_2 concentrations at the CUH in Seattle compared to Forks is evident in the
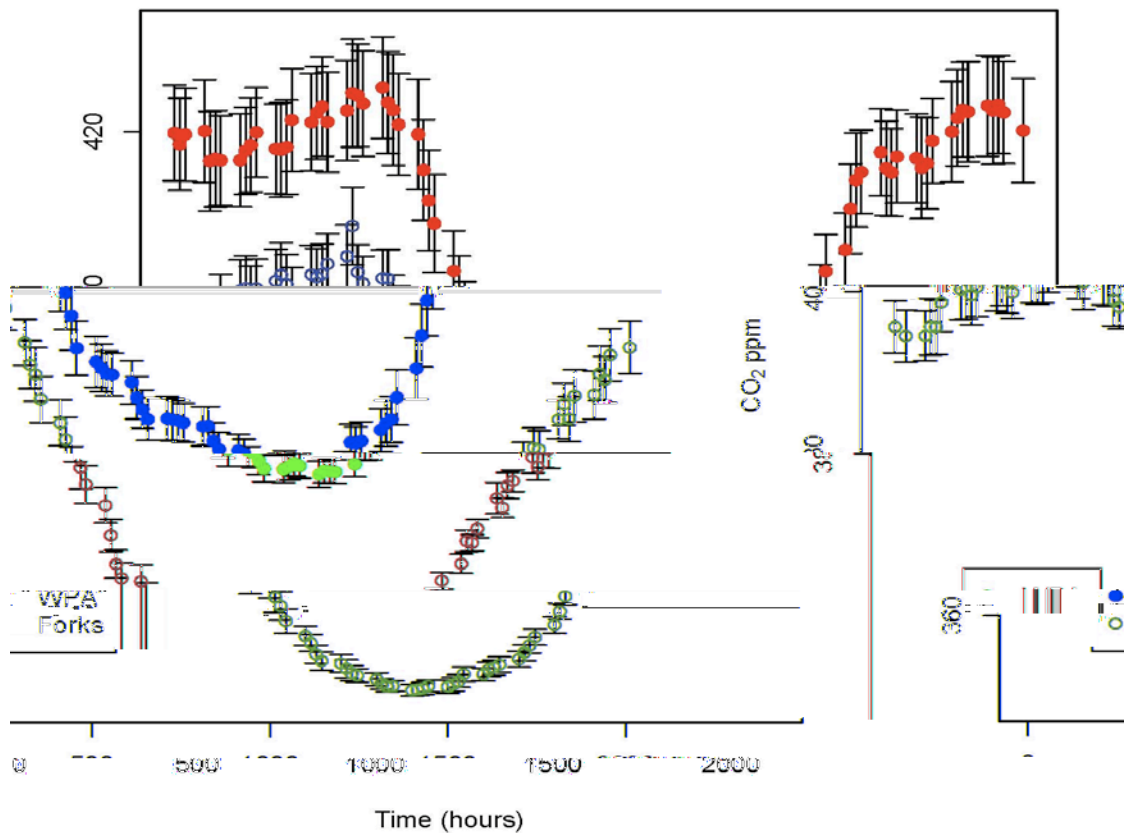
diurnal patterns displayed in Fig. 3. The CO_2 concentrations at the CUH

averaged 8.1 % higher than concentrations at Forks.
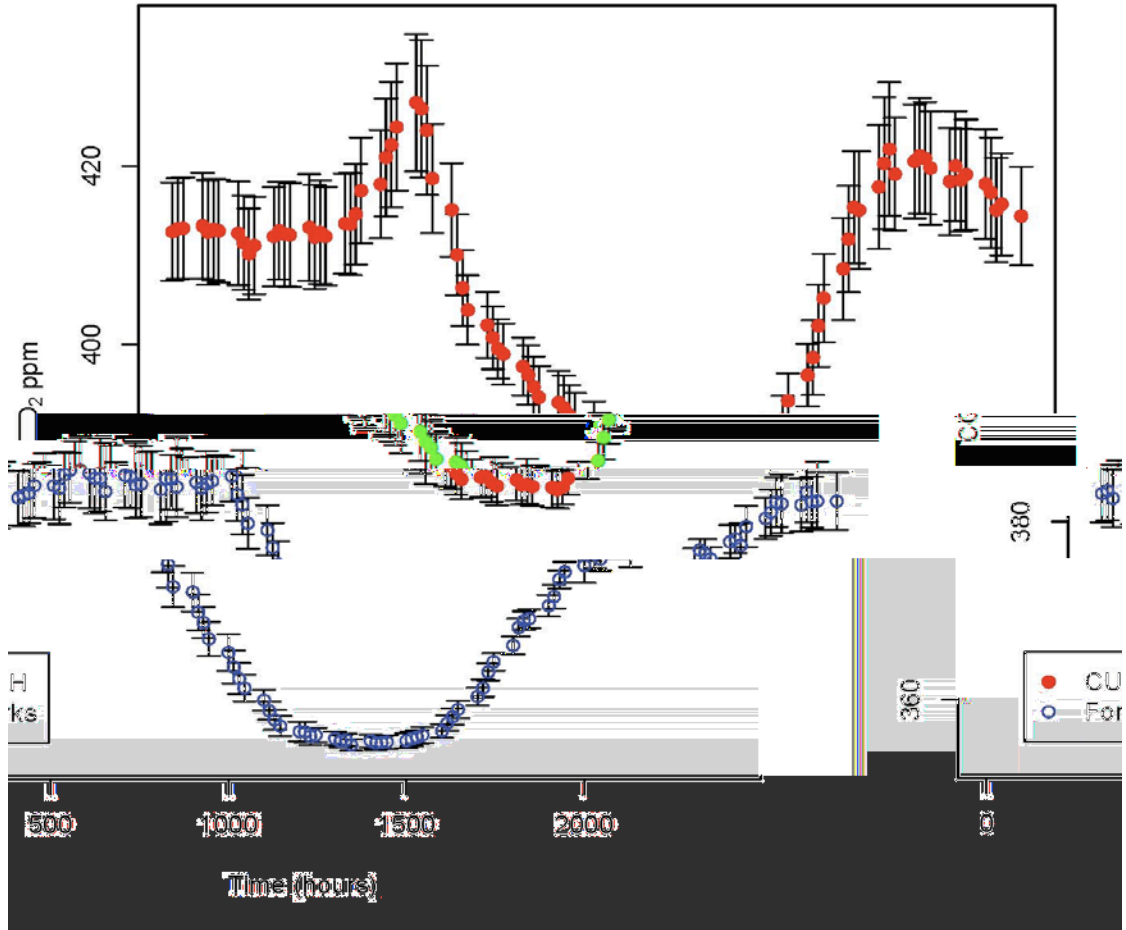
Fig. 3 Near surface CO_2 concentrations averaged over a 24-hour period at CUH in Seattle and the rural site in Forks from September 25th through October 21st, 2008. Error bars represent standard error for all graphs.

In the early morning the highest average CO_2 concentrations were

reached, at the WPA this value was 425 ppm at 600 hours (6:00 am) while the

maximum average concentration at Forks was 408 ppm at 515 hours (5:15

am). Minimum average CO_2 concentrations were found during the afternoon

hours, at the WPA this value was 381 ppm at 1630 hours (4:30 pm) while at Forks the minimum average CO_2 concentration was 350 ppm at 1400 hours (2:00pm). The CO_2 concentrations at the WPA averaged 5.6 % higher than concentrations observed at Forks during the four week sampling period (Fig. 4).

Fig. 4 Near surface CO_2 concentrations averaged over a 24-hour period at WPA and Forks from September 5th through September 22nd, 2008.

The typical diurnal pattern of CO_2 is not as evident in the chambers due to the small amount and size of the vegetation, i.e. the effects of respiration and photosynthesis are not exerting as strong of control over the concentration

of CO_2 as they do in natural and field settings. There is a clear distinction

between the average elevated and ambient concentrations of CO_2 (referred to

as [E]-CO_2 and [A]-CO_2, respectively, for the rest of this document) that were

recorded in the chambers. In the controlled environment the two [E]-CO_2

chambers had an average of 740 ppm for the duration of the experiment while

the two [A]-CO_2 chambers averaged 460 ppm (Fig. 5).
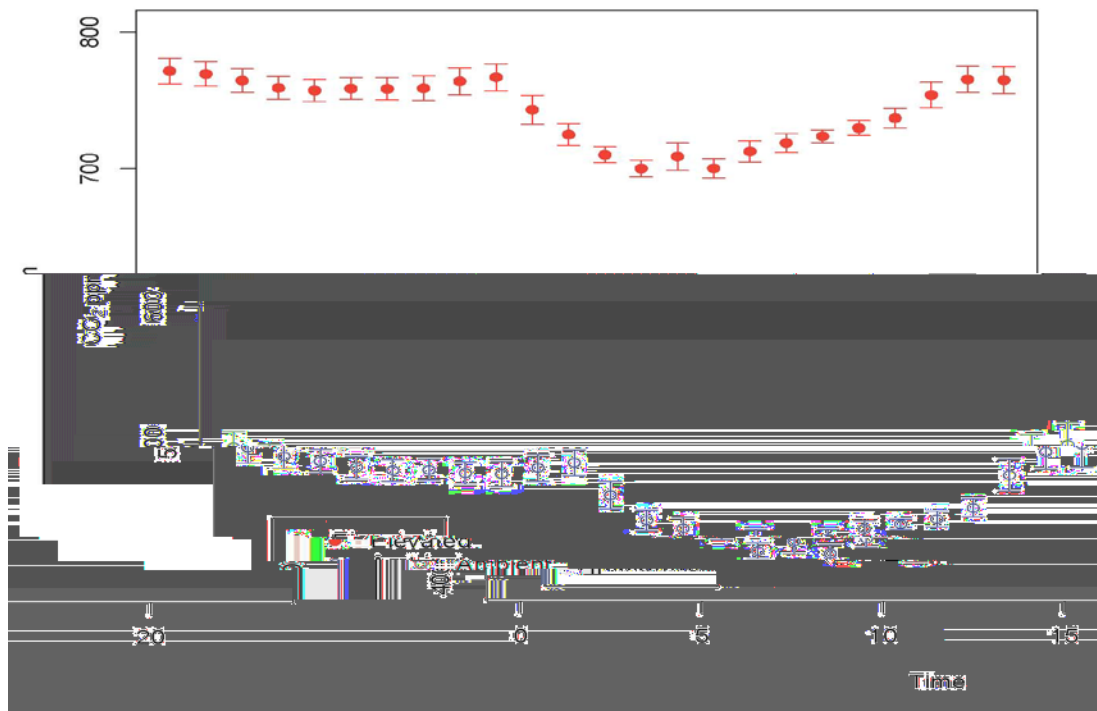
Fig. 5: CO_2 concentrations for growth chambers in the controlled portion of the experiment from January 25th- March 3rd, 2009. [E]-CO_2 and [A]-CO_2 concentrations are averaged over a 24-hour period for the two chambers exposed to each level.

The air temperature at the CUH site was higher on average than at

Forks. The greatest differences were found in the afternoon at 1615 hours

(4:15 pm) when temperatures at CUH averaged 3°C higher than Forks.

Nighttime temperatures at CUH also averaged 2°C higher than Forks at 0

["
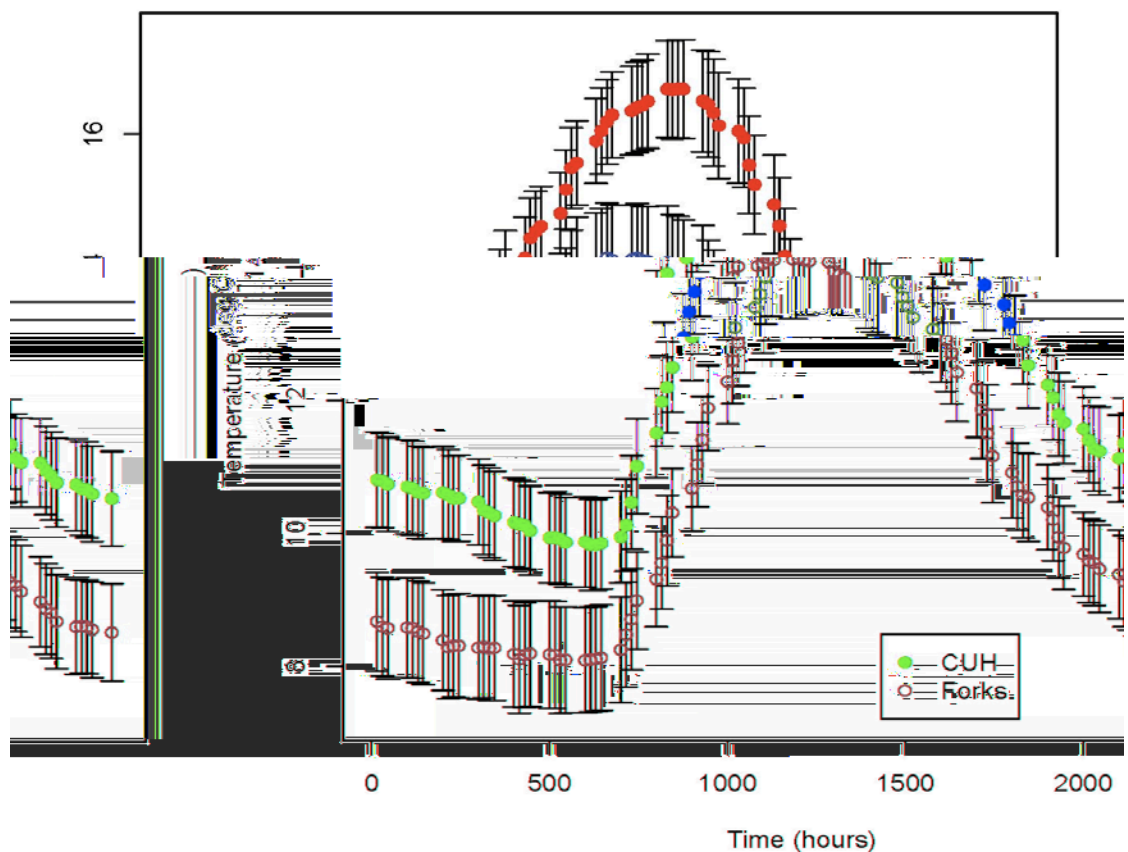
Fig. 7: Air temperature averaged over a 24-hour time period at WPA in Seattle and the rural site in Forks from September 5th through September 22nd, 2008.

Temperatures within the chambers remained constant for the duration of the controlled experiment. The average temperature for all four chambers was 21.4°C with a standard error of ±0.07.

Light levels at the CUH and Forks followed the same pattern and were measured in the form of Photosynthetic Photon Flux (PPF), which correlates with the amount of Photosynthetically Active Radiation (PAR) the plants have

available to them. Figure 8 shows a diurnal light pattern typical of open areas

with values close to zero overnight and a steady increase in light from 600

hours (6:00 am, sunrise) to a peak around noon and a steady decline until

1830 hours (6:30 pm, sunset).
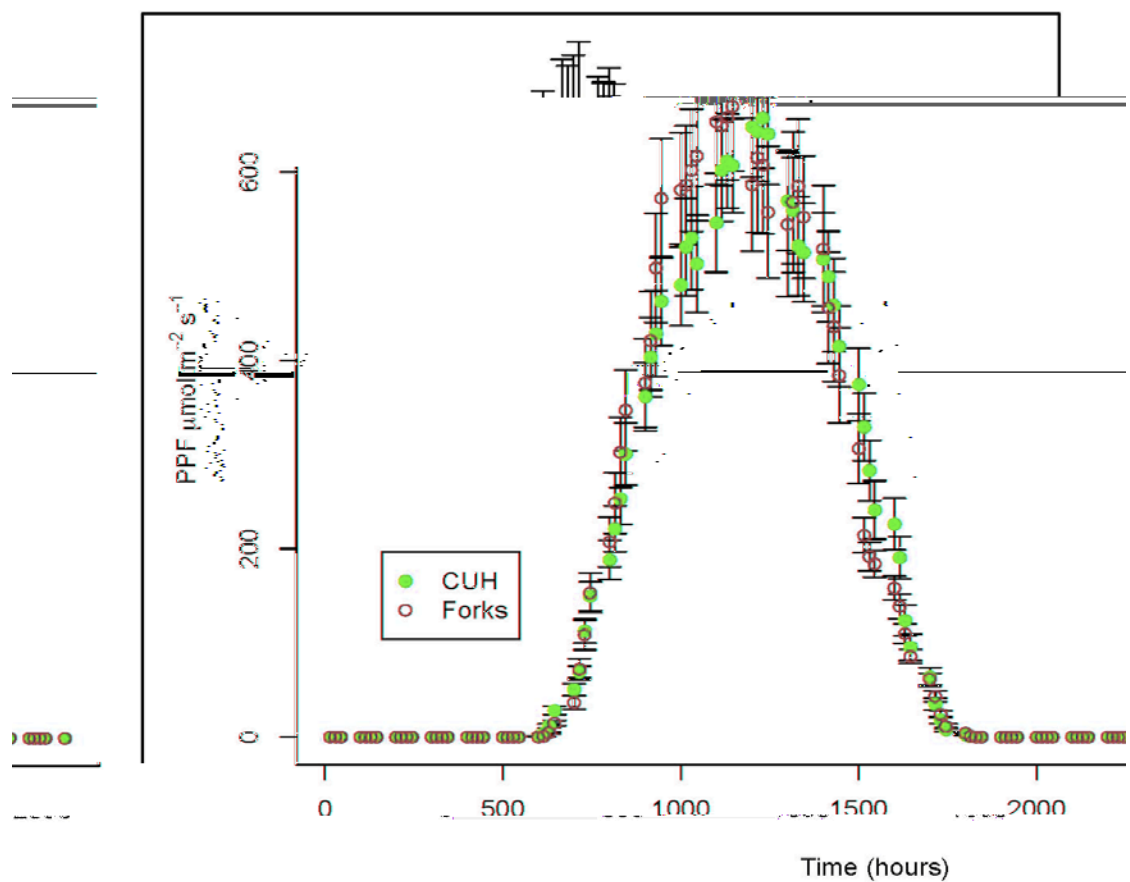
Fig. 8: Light levels averaged over a 24-hour time period at the CUH in Seattle and the rural
site in Forks from September 25th through October 21st, 2008.

Light conditions at the SDOT, WPA, UWAA sites were lower than those

at Forks and CUH due to building and tree obstructions overhead during some

parts of the day. The hemispherical photo analysis confirmed the similarities

in light conditions between Forks and the CUH that the quantum sensor

recorded (Fig. 8) and reveals the **Table 1**: Percent openness for each · at the
site.

SDOT and WPA sites. The UWA   ,   ,   ιness

compared to the other four sites indicating shaded conditions there throughout

the day (Table 1).

| Site	% Openness
Forks	79.31
CUH	75.96
SDOT	47.97
WPA	34.54
UWAA	12.78

Generally, diurnal relative humidity patterns are similar to diurnal CO_2

patterns. Maximum values are observed in early morning and late night hours

due to plant respiration and moisture condensation. Minimum values are

typically found during mid-day when air temperatures are high. Relative

humidity levels were higher at Forks compared to the CUH, with the most

significant differences observed over night and mid-day (Fig. 10).
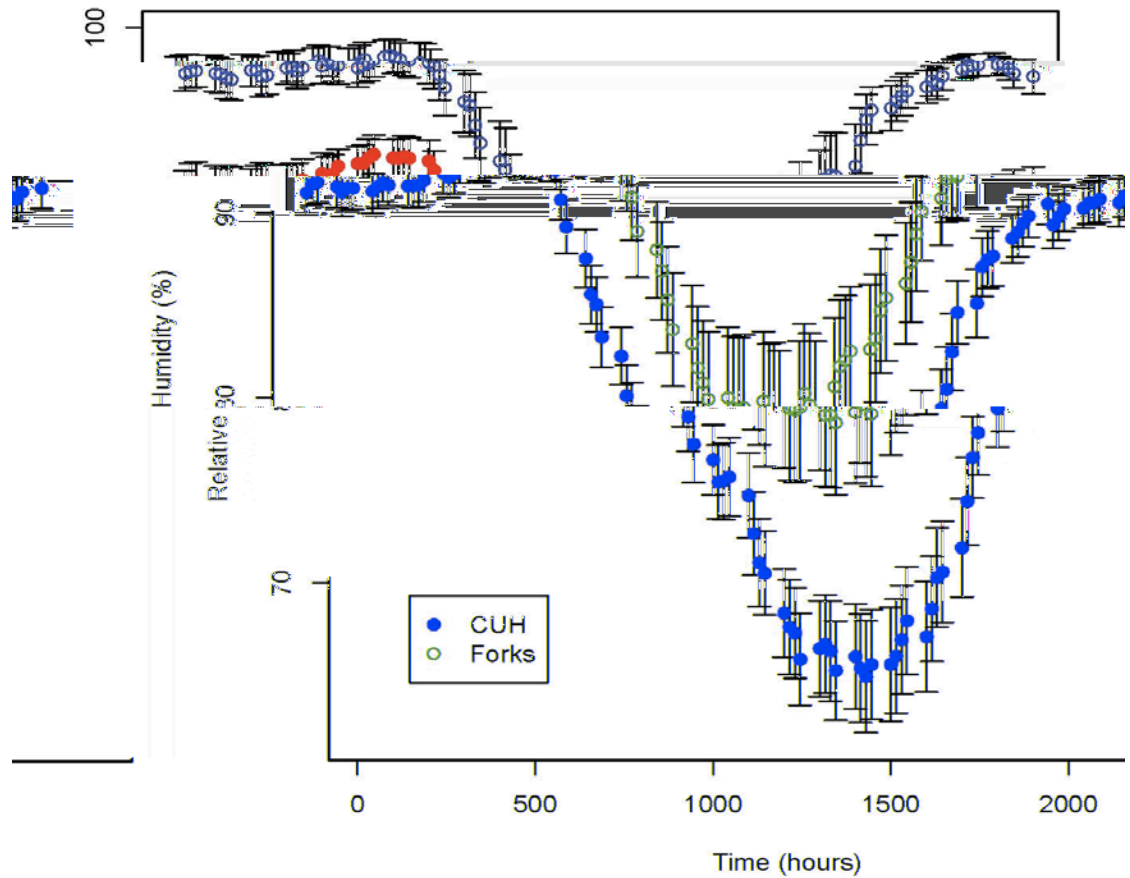
Fig. 10 Percent relative humidity at the CUH in Seattle and the rural site in Forks averaged over a 24-hour time period from September 25th through October 21st, 2008

The relative humidity values at the WPA and Forks sites did not differ to the degree observed between CUH and Forks. The difference in percent relative humidity between WPA and Forks resembles the trend found for air-temperature comparisons made between said locations (Fig. 11).
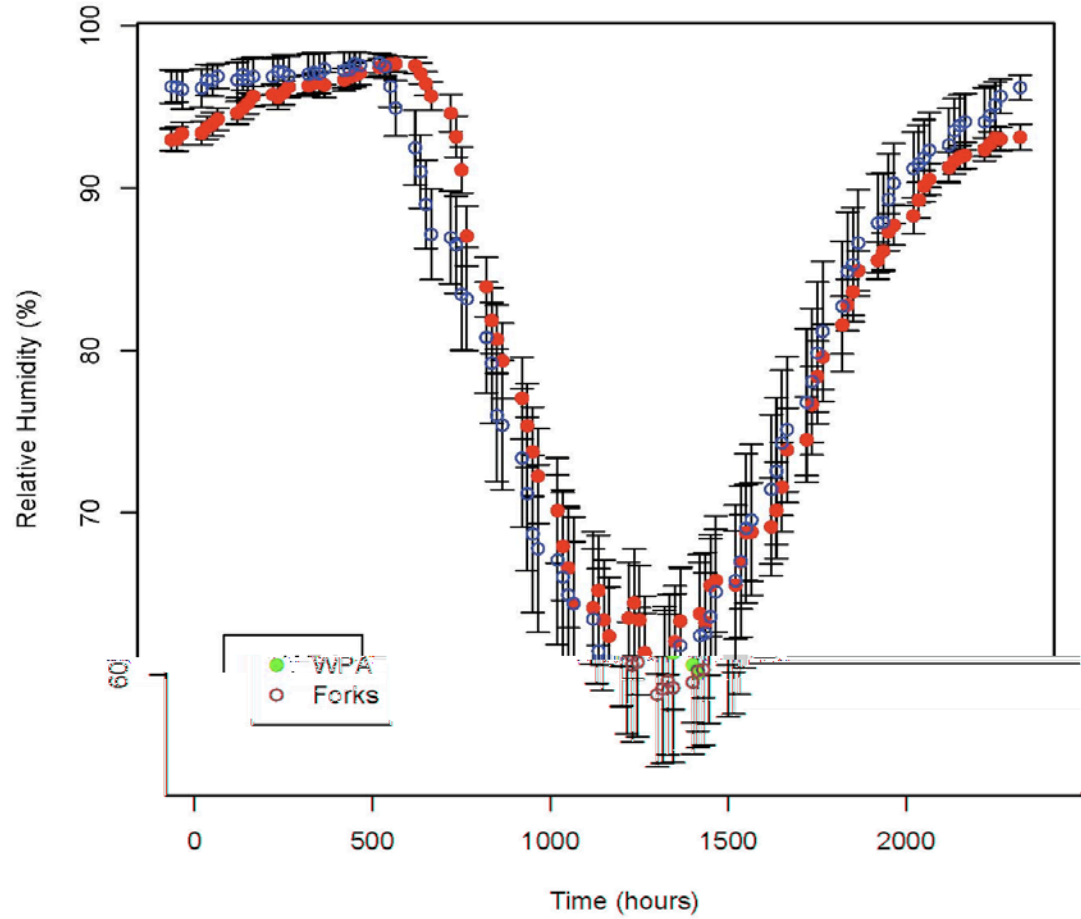
Fig. 11 Percent relative humidity at the Washington Park Arboretum (WPA) in Seattle and the rural site in Forks averaged over a 24-hour time period from September 5th through September 22nd, 2008.

3.2 Arabidopsis thaliana (field study)

At all sites in Seattle, *A.thaliana* (Col-0 ecotype) plants exhibited lower stomatal numbers on average compared to those grown in Forks. The average stomatal density (SD) of Col-0 plants grown in Forks was 266.9 with a standard error of ± 11.7. The SD of Col-0 plants grown at UWAA were the lowest compared to the rest of the sites with an average of 119.2 ± 10 while the most variability was seen in plants grown at WPA (Fig. 12).
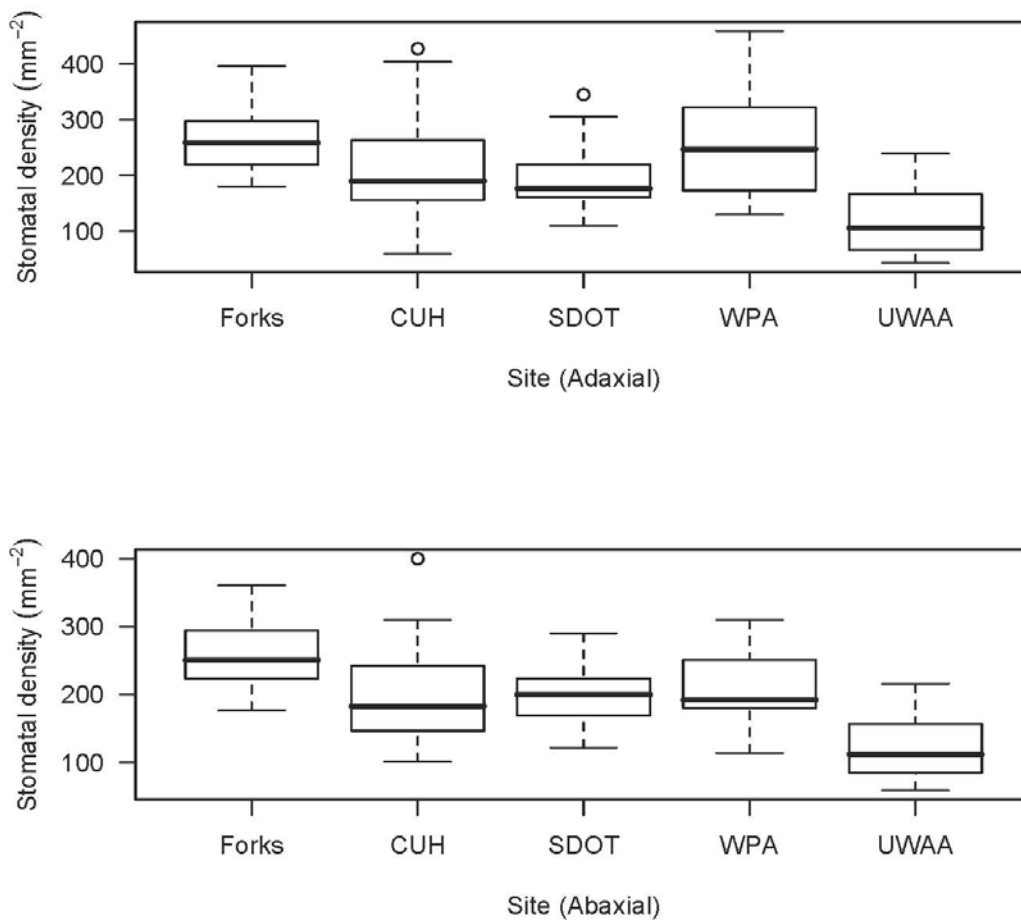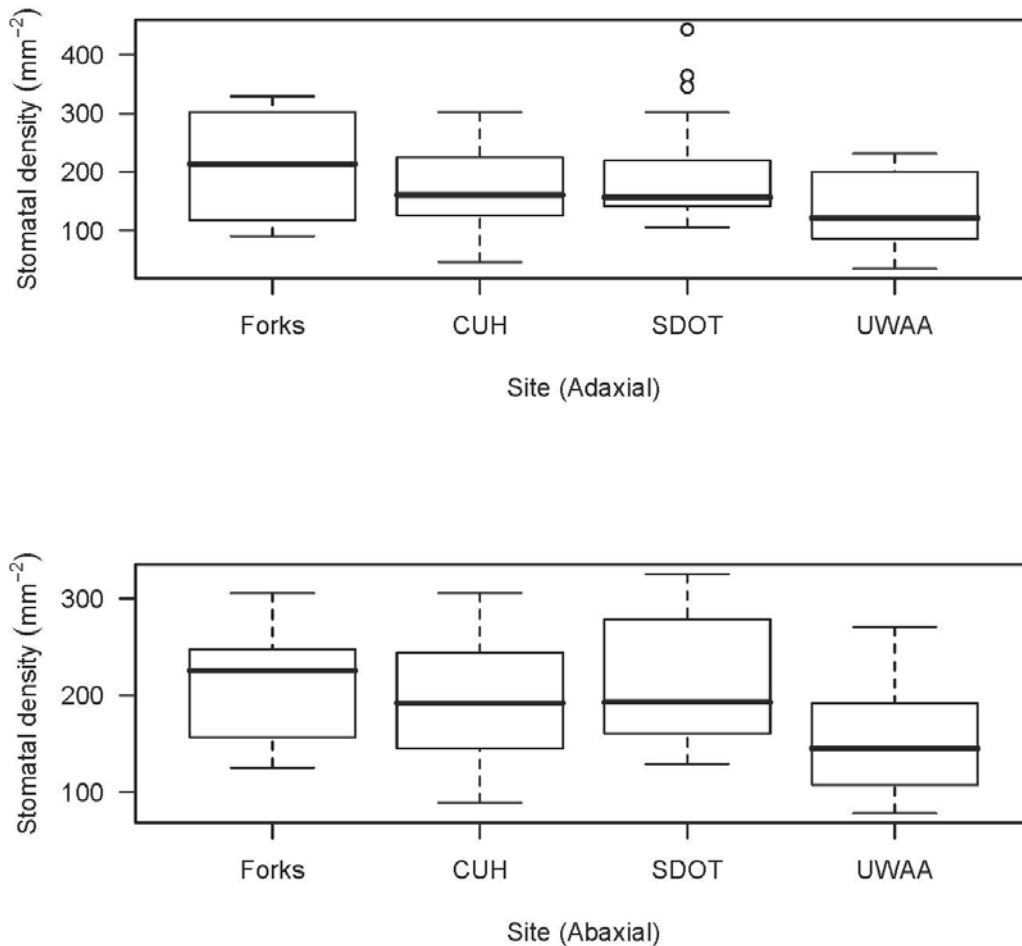
Fig. 12 Average stomatal density for both adaxial and abaxial surfaces of *A. thaliana* plants (Col-0 ecotype) grown at all five sites.

Epidermal cell density (ED) of *A. thaliana* plants (Col-0 ecotype) also differed among sites. ED of the abaxial surface is higher for plants grown at Forks than for those grown in Seattle while it varied for the adaxial surface of leaves compared between the sites (Fig. 13).
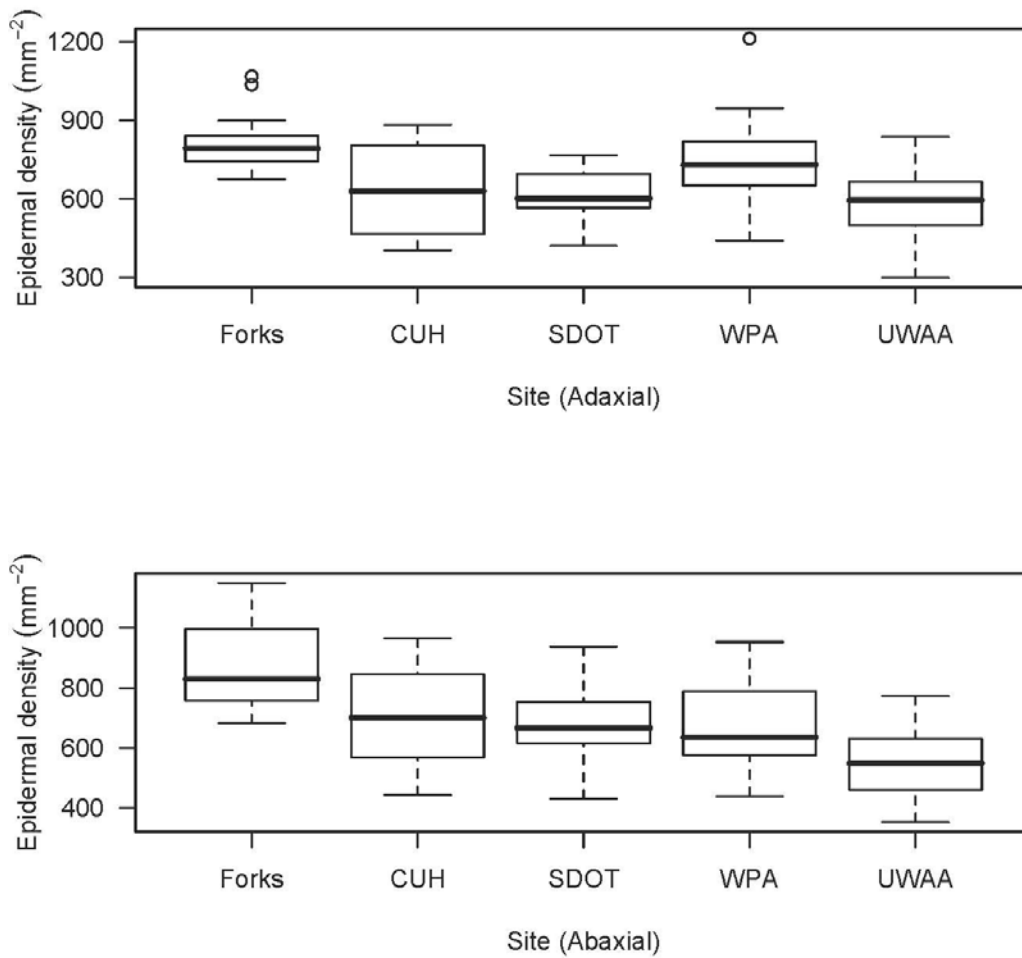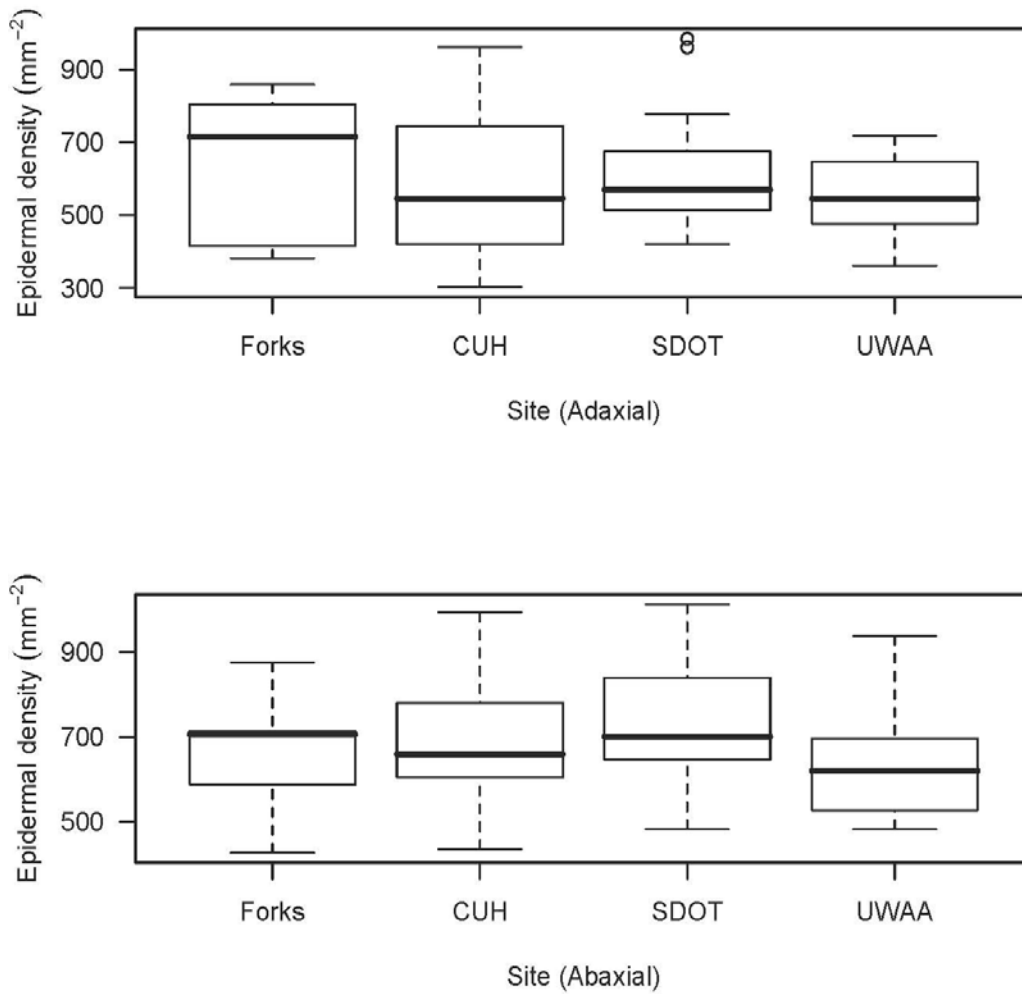

Fig. 13 Average epidermal density for both adaxial and abaxial surfaces of A. thaliana plants (Col-0 ecotype) grown at all five sites.

The average SD for the adaxial surface of the Ws ecotype was greatest at Forks compared to that of Ws plants grown at CUH, SDOT and UWAA. More variability in average SD was found on the abaxial surface of Ws plants grown at all of the sites (Fig. 14). Stomatal numbers from the WPA site are not included due to mortality.

Fig. 14 Average stomatal density for both adaxial and abaxial surfaces of A. thaliana plants (Ws ecotype) grown at all five sites.

The epidermal density (ED) for Ws plants grown at the sites follows the same trends as the SD of Ws plants for both surfaces (Fig. 15).

Fig. 15 Average epidermal density for both adaxial and abaxial surfaces of *A. thaliana* plants (Ws ecotype) grown at all five sites.

Statistical comparisons of the average stomatal and epidermal density between the sites provide more detail about how and where the significant differences in these numbers lie (Table 2).   The lowest *p*-value, i.e. the most significance, was found for the comparison made between stomatal density (SD) of Col-0 plants grown at Forks and UWAA. At UWAA a 50%+ decrease in SD was observed for both surfaces of Col-o plants compared to Forks. Significant differences in SD of Col-0 plants were also found in comparisons made between Forks and Seattle sites (CUH and WPA) on the abaxial surfaces and at SDOT for both surfaces. The epidermal density (ED) is significantly higher for Col-0 plants grown at Forks compared to those grown in Seattle for both surfaces with percent decreases in ED ranging between 6.94% and 34.7 % (Table 2).

Table 2 Stomatal density (SD) and epidermal density (ED) of *Arabidopsis thaliana* grown in a rural and four urban sites. *P* represents the probability of paired t-test between Forks (rural) and each of the urban sites in Seattle.

Ecotype (Surface)	Site (n)	SD±SE (mm^{-2})	(%)	*p*-value, a(2) (SD)	ED (mm^{-2})	(%)	*p*-value, α(2) (ED)
Col-0 (Adaxial)	Forks (5)	266.9 ± 11.7			804.1 ± 18.8		
	CUH (4)	216.2 ± 18.3	-18.9	0.1<p<0.2	630.6 ± 31	-21.6	0.01<p<0.02
	SDOT (5)	192.6 ± 11.5	-27.8	0.02<p<0.05	609 ± 18.8	-24.3	0.002<p<0.005
	WPA (3)	263.2 ± 26.9	-1.4	0.2<p<0.5	748.3 ± 42.4	-6.94	0.2<p<0.5
	UWAA (4)	119.2 ± 10	-55.3	0.002<p<0.005	583.7 ± 21.7	-27.4	0.002<p<0.005
Col-0 (Abaxial)	Forks	260.8 ± 9.3			864.4 ± 25.7		
	CUH	197.2 ± 13.3	-24.4	0.005<p<0.01	709.4 ± 29.9	-17.9	0.02<p<0.05
	SDOT	202.6 ± 7.9	-22.3	0.01<p<0.02	676.6 ± 20.5	-21.7	0.01<p<0.02
	WPA	211.8 ± 15.1	-18.8	0.02<p<0.05	668.1 ± 38.9	-22.7	0.02<p<0.05
	UWAA	120.6 ± 7.8	-53.7	p<0.001	564.1 ± 19.7	-34.7	0.002<p<0.005
Ws (Adaxial)	Forks (2)	216.9 ± 29.2			650.9 ± 58.9		
	CUH (4)	172.9 ± 14.3	-20.3	0.2<p<0.5	567.1 ± 41.9	-13	0.5<p
	SDOT (4)	191.5 ± 15.4	-11.7	0.5<p	600.3 ± 25.4	-7.8	0.5<p
	UWAA (3)	136.7 ± 16.7	-36.9	0.1<p<0.2	554.2 ± 30.8	-15	0.5<p
Ws (Abaxial)	Forks	211.8 ± 17.9			670.9 ± 38.2		
	CUH	191.2 ± 13.8	-9.7	0.5<p	682.1 ± 33.9	1.7	0.5<p
	SDOT	214 ± 11.5	1	0.5<p	730.9 ± 23.9	8.9	0.2<p<0.5
	UWAA	150.6 ± 14.9	-28.9	0.1<p<0.2	642.1 ± 37.5	-4.3	0.5<p

SD, stomatal density; SE, standard error; ED, epidermal density; %, Percent increase or decrease compared to Forks.

The Ws ecotype had fewer pair-wise comparisons between sites with significant differences in stomatal density (SD). The percent decrease in SD for Ws plants grown in Seattle compared to Forks is higher on the adaxial surfaces than the abaxial. However, the most significant differences in SD was found between Forks and the UWAA for both leaf surfaces at $0.1 < p < 0.2$ (Table 2). The ED for the Ws ecotype is highest on the adaxial surface of plants grown in Forks compared to Seattle. On the abaxial surface SDOT has the highest ED at 730.9 ± 23.9 which is 8.9% higher than Forks (Table 2). None of the ED values for Ws plants grown in Seattle were significantly different than plants grown in Forks.

Overall, stomatal index (SI) does not differ much among Col-0 plants grown at all sites except for UWAA, which remains low (Fig. 16). However, a closer look reveals the SI for the abaxial surface of Col-0 plants grown at CUH and both surfaces of plants grown at UWAA is significantly lower than SI for plants grown at Forks (Table 3). Overall, SI of the Ws ecotype was similar to the SI of Col-0 (Fig. 17). However, significance between SI of Ws plants was only found in comparisons made between Forks and UWAA where the SI of plants grown at Forks was 20.8% higher than UWAA.
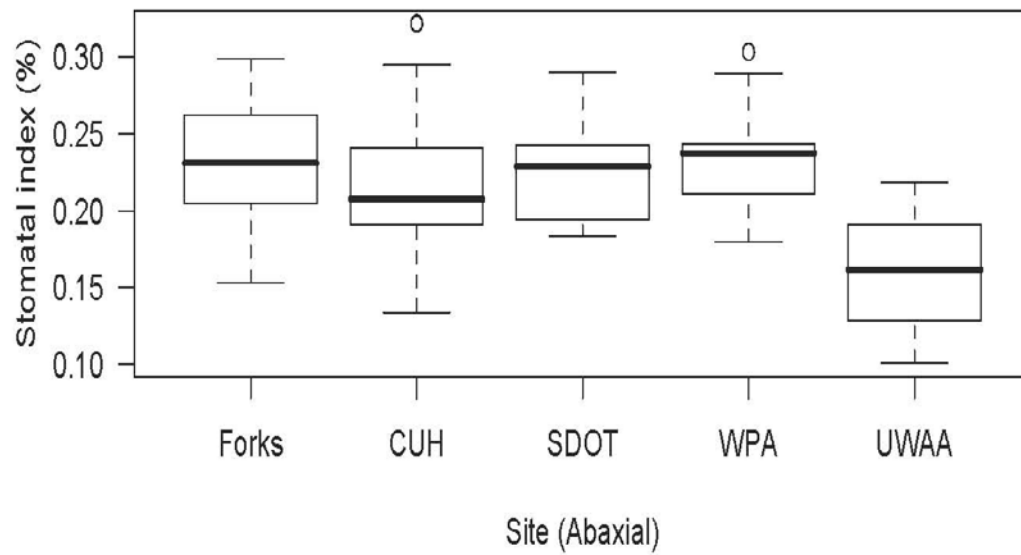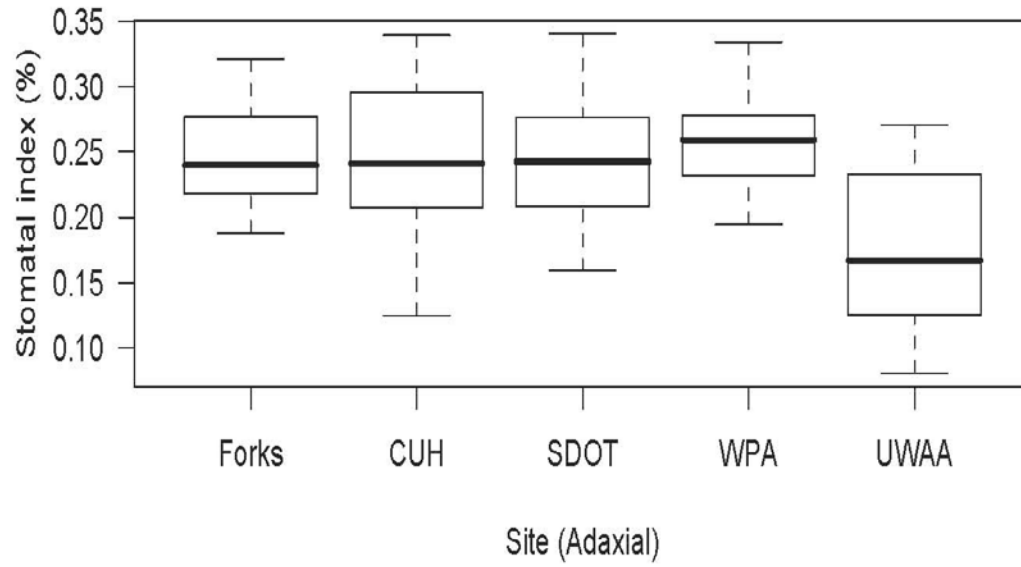
Fig. 16 Average stomatal index for both adaxial and abaxial surfaces of *A. thaliana* plants (Col-0 ecotype) grown at all five sites.
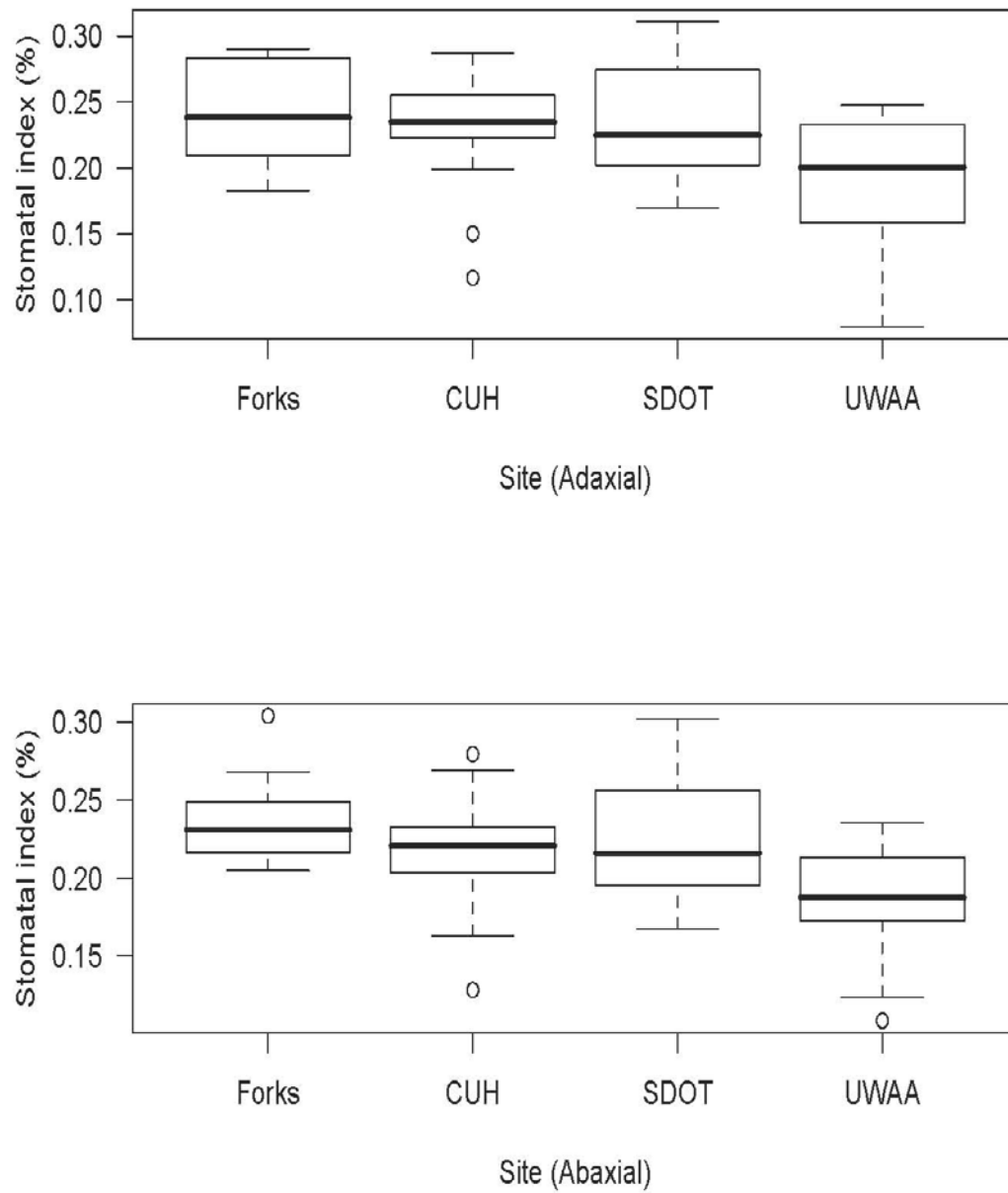
Fig. 17 Average stomatal index for both adaxial and abaxial surfaces of *A. thaliana* plants (Ws ecotype) grown at all five sites.

Table 3 Stomatal index (SI) of *Arabidopsis thaliana* grown in a rural and four urban sites. *P* represents the probability of paired t-test between Forks (rural) and each of the urban sites in Seattle.

| Ecotype (Surface)	Site (n)	SI± SE	*p*-value, a(2) (SI)
Col-0 (Adaxial)	Forks (5)	0.247 ± 0.007	
	CUH (4)	0.249 ± 0.011	0.5 < *p*
	SDOT (5)	0.243 ± 0.008	0.5 < *p*
	WPA (3)	0.259 ± 0.009	0.5 < *p*
	UWAA (4)	0.177 ± 0.010	0.001<p<.002
Col-0 (Abaxial)	Forks	0.233 ± 0.007	
	CUH	0.216 ± 0.008	0.05<p<0.1
	SDOT	0.226 ± 0.006	0.2<p<.5
	WPA	0.234 ± 0.009	0.1<*p*<0.2
	UWAA	0.161 ± 0.006	*p*<.001
Ws (Adaxial)	Forks (2)	0.241 ± 0.012	
	CUH (4)	0.232 ± 0.009	0.5 < *p*
	SDOT (4)	0.235 ± 0.008	0.5 < *p*
	UWAA (3)	0.189 ± 0.015	0.02<*p*<0.05
Ws (Abaxial)	Forks	0.238 ± 0.009	
	CUH	0.216 ± 0.008	0.2<*p*<.5
	SDOT	0.224 ± 0.007	0.5 < *p*
	UWAA	0.186 ± 0.010	0.02<*p*<0.05

SI, stomatal index; SE, standard error.

3.3 Arabidopsis thaliana (chamber study)

A. thaliana (Col-0 ecotype) exhibited a higher SD for plants grown in [A]-CO_2 chambers compared to those grown in [E]-CO_2 chambers on both surfaces of the leaf (Fig. 18). However, the difference in SD was most significant when compared between the abaxial surfaces of leaves (Table 4).

Table 4 Stomatal density (SD), epidermal density (ED), and stomatal index of *Arabidopsis thaliana* grown in ambient and elevated CO_2 chambers. *P* represents the probability of paired t-test between ambient and elevated CO_2 chambers.

Ecotype (Surface)	CO_2 (n)	SD± SE (mm^{-2})	p-value, α(2) (SD)	ED± SE (mm^{-2})	p-value, α(2) (ED)	SI± SE	p-value, α(2) (SI)
Col-0 (Adaxial)	[A]-CO_2 (6)	136.7 ± 10.8		447.2 ± 22.9		0.229 ± 0.007	
	[E]-CO_2 (5)	110 ± 10.9	0.1< p <0.2	352 ± 14.5	0.2< p <0.5	0.231 ± 0.011	p >0.5
(Abaxial)	[A]-CO_2	140.8 ± 8.6		516.5 ± 23.5		0.211 ± 0.006	
	[E]-CO_2	110.5 ± 9.6	0.02<p< 0.05	429.1 ± 16.7	0.2< p <0.5	0.200 ± 0.009	0.2< p <0.5
Ws (Adaxial)	[A]-CO_2 (7)	111.8 ± 6.8		438.9 ± 20.9		0.202 ± 0.006	
	[E]-CO_2 (8)	102 ± 5.2	0.2< p <0.5	358.6 ± 14.3	0.1< p <0.2	0.219 ± 0.005	0.05< p <0.1
(Abaxial)	[A]-CO_2	140.3 ±5.8		520.3 ± 17.9		0.213 ± 0.005	
	[E]-CO_2	122.2 ±5.6	0.02<p< 0.05	445.4 ± 18.6	0.05< p <0.1	0.215 ± 0.004	p >0.5

SD, stomatal density; SI, stomatal index; ED, epidermal density; [A]-CO_2, ambient CO_2; [E]-CO_2, elevated CO_2;
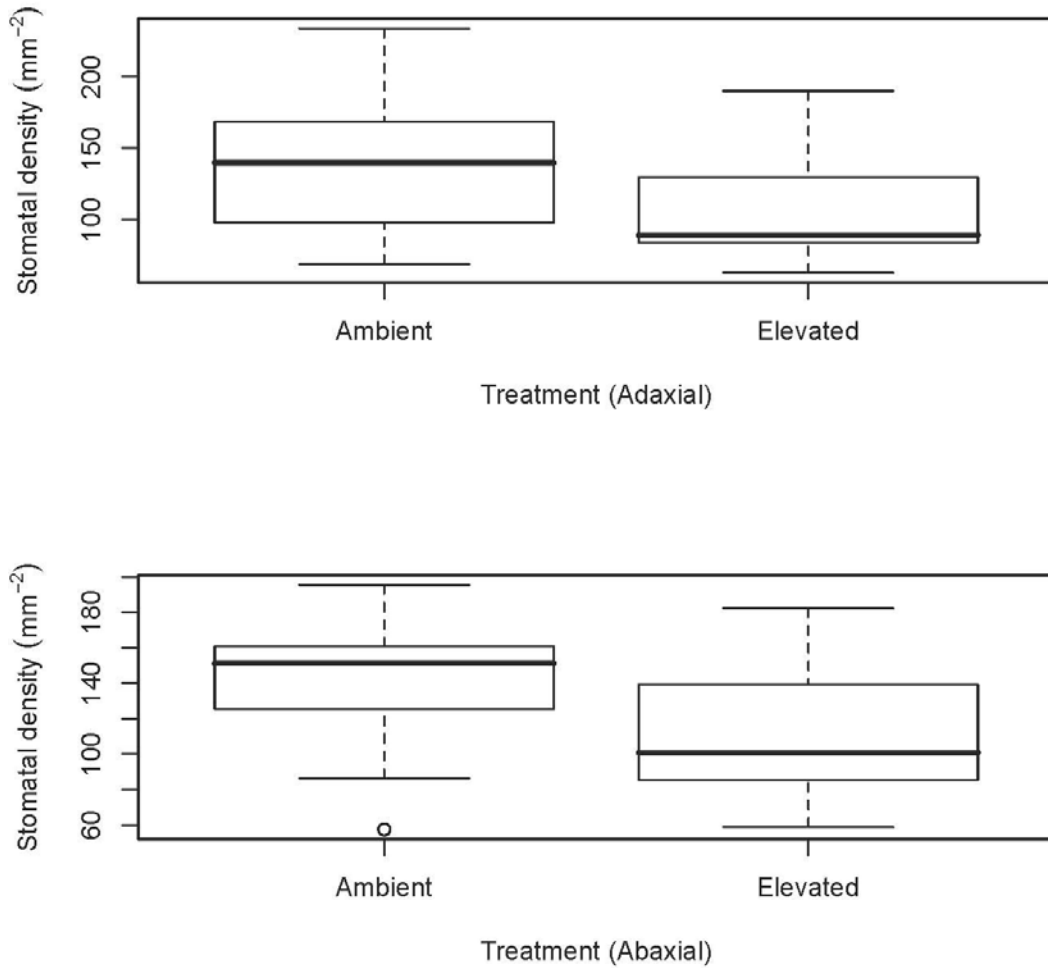
Fig. 18 Average stomatal density for both adaxial and abaxial surfaces of A. thaliana plants (Col-0 ecotype) grown in ambient and elevated CO_2 chambers.

The epidermal density (ED) was slightly lower on average for the adaxial surface of Col-0 leaves grown in [E]-CO_2 chambers (Fig. 19) but differences weren't significant for either surface (Table 4).
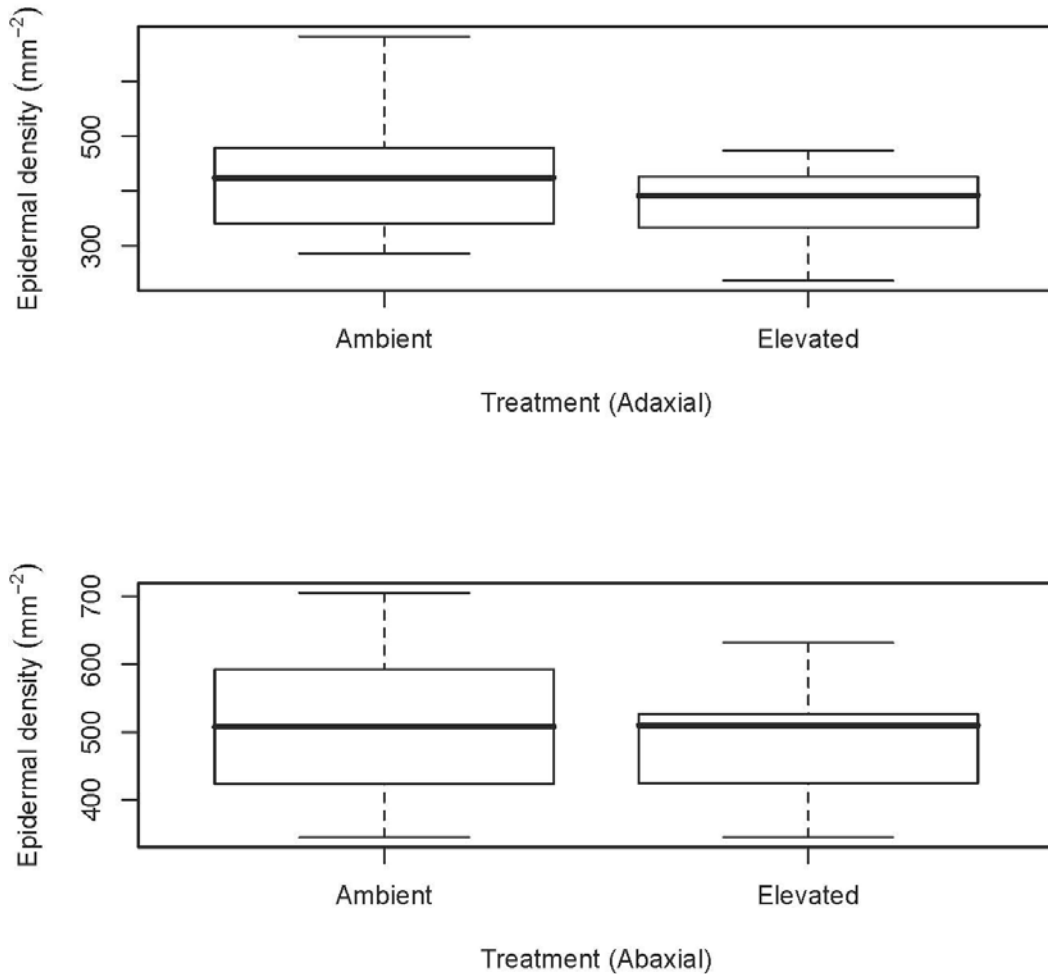
Fig. 19 Average epidermal density (ED) for both adaxial and abaxial surfaces of A. thaliana plants (Col-0 ecotype) grown in ambient and elevated CO_2 chambers.

The stomatal index (SI) on both surfaces of the Col-0 ecotype was lower for leaves grown under [E]-CO_2 concentrations compared to those grown in ambient [A]-CO_2 chambers (Fig. 20). However, according to the statistical analysis, these differences are not significant (Table 4).

Fig. 20 Average stomatal index for both adaxial and abaxial surfaces of A. thaliana plants (Col-0 ecotype) grown in ambient and elevated CO_2 chambers.

The average stomatal density (SD) for both surfaces of A. thaliana (Ws ecotype) plants grown in [E]-CO_2 chambers is lower than those grown in [A]-CO_2 chambers (Fig. 21).   However, only the abaxial surfaces of these plants prove to exhibit significantly lower SD (Table 4).
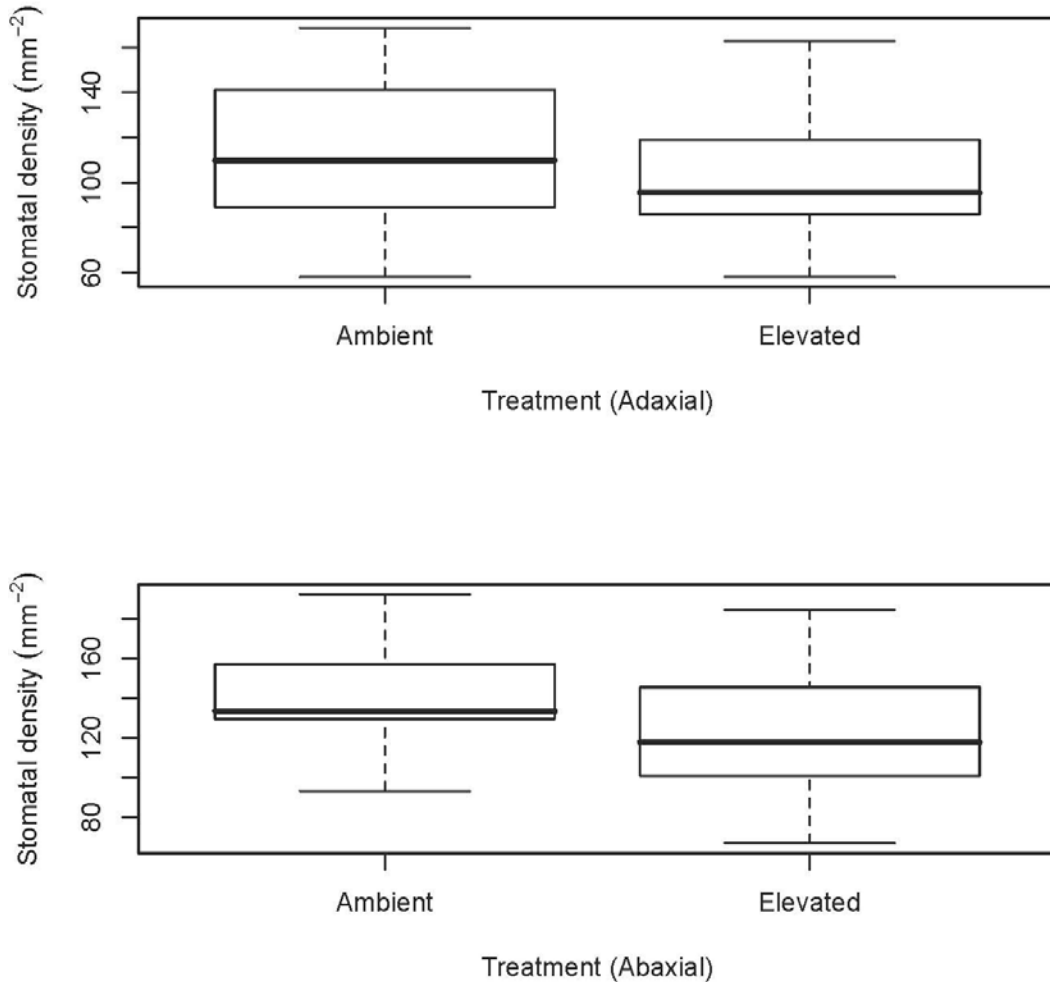
Fig. 21 Average stomatal density for both adaxial and abaxial surfaces of A. thaliana plants (Ws ecotype) grown in ambient and elevated CO_2 chambers.

The average ED for Ws plants follows the same trend as SD with lower values for both leaf surfaces grown in $[E]$-CO_2 chambers compared to those grown in $[A]$-CO_2 chambers (Fig. 22). These differences are only significant for the comparison between the abaxial surfaces of leaves (Table 4).
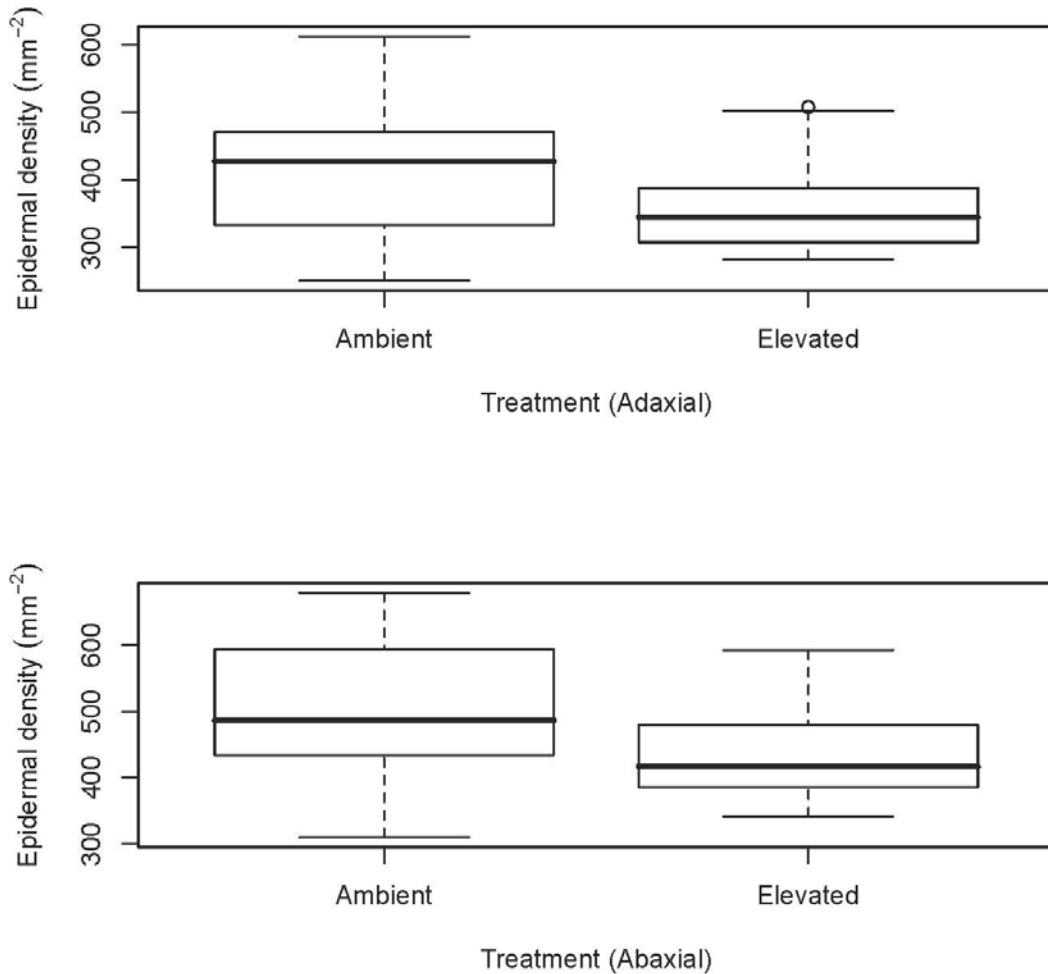
Fig. 22 Average epidermal density for both adaxial and abaxial surfaces of *A. thaliana* plants (Ws ecotype) growing in ambient and elevated CO_2 chambers.

The average SI for Ws plants has the opposite trend of SD with higher values for plants grown in [E]-CO_2 chambers compared to those grown in [A]-CO_2 chambers (Fig. 23). This difference is significant on the adaxial surface of leaves (Table 4).
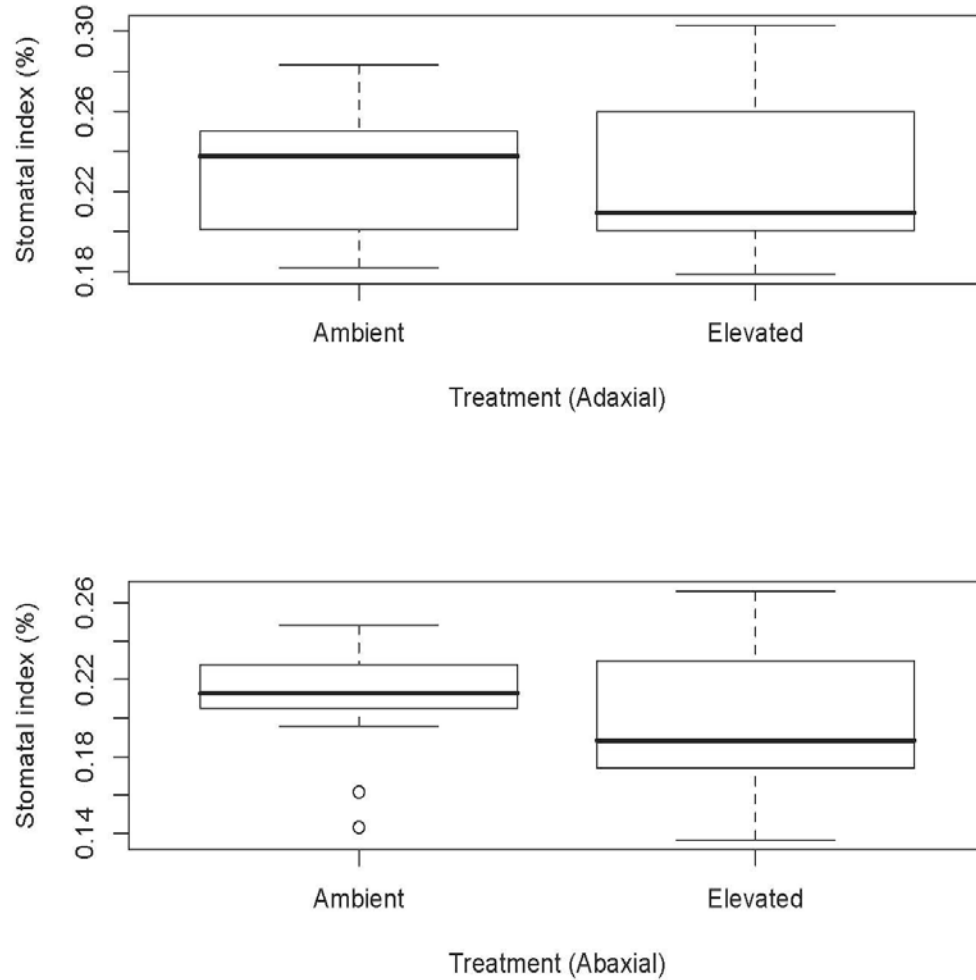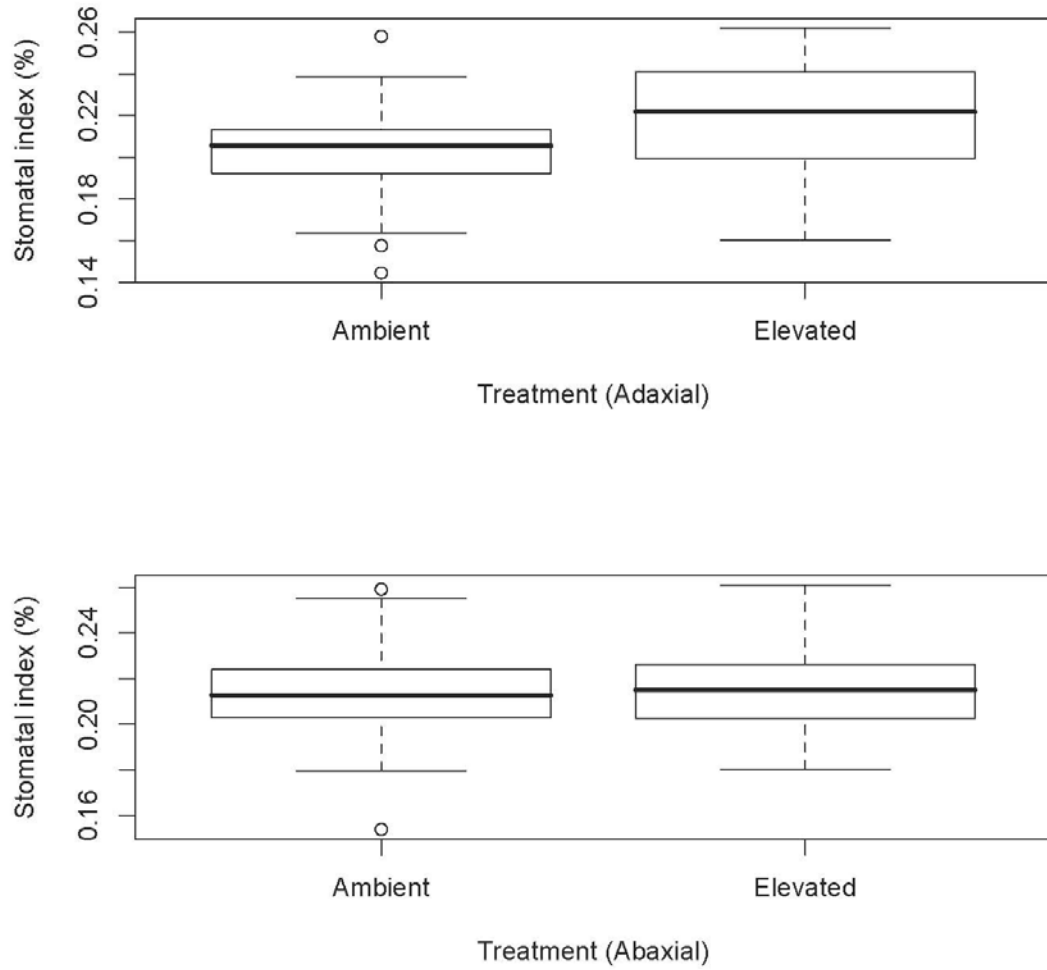
Fig. 23 Average stomatal index (SI) for both adaxial and abaxial surfaces of *A. thaliana* plants (Ws ecotype) growing in ambient and elevated CO_2 chambers.

Results show that poplar hybrids growing at these sites had similar stomatal numbers. There was no significant difference found among the stomatal numbers of leaves that developed after the plants had been growing at each of the sites (Figs. 31 & 32). In addition, there was no significant difference in stomatal numbers between the mature leaves that had already formed before being brought to each site and those that developed while exposed to site conditions.
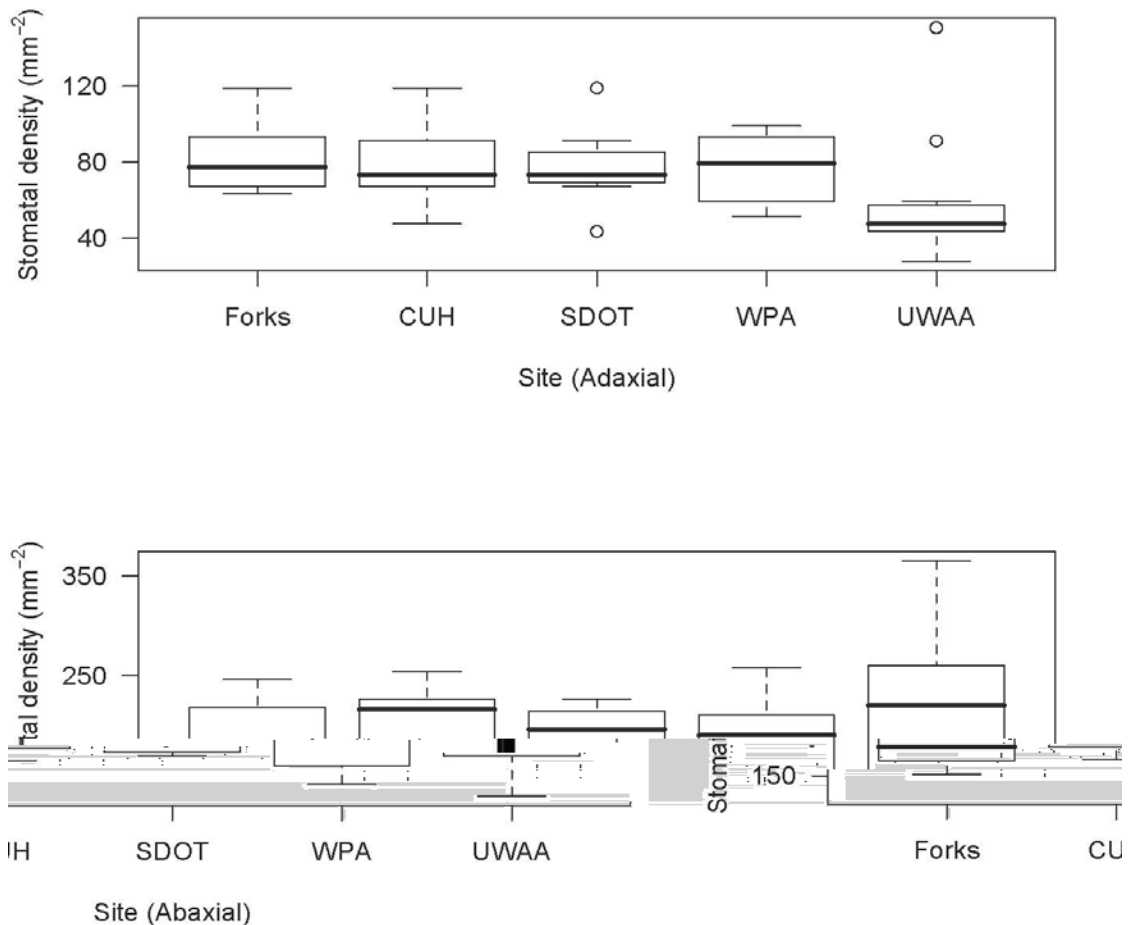
Fig. 31 Average stomatal density for both adaxial and abaxial surfaces of leaves for poplar hybrid plants grown at each site.
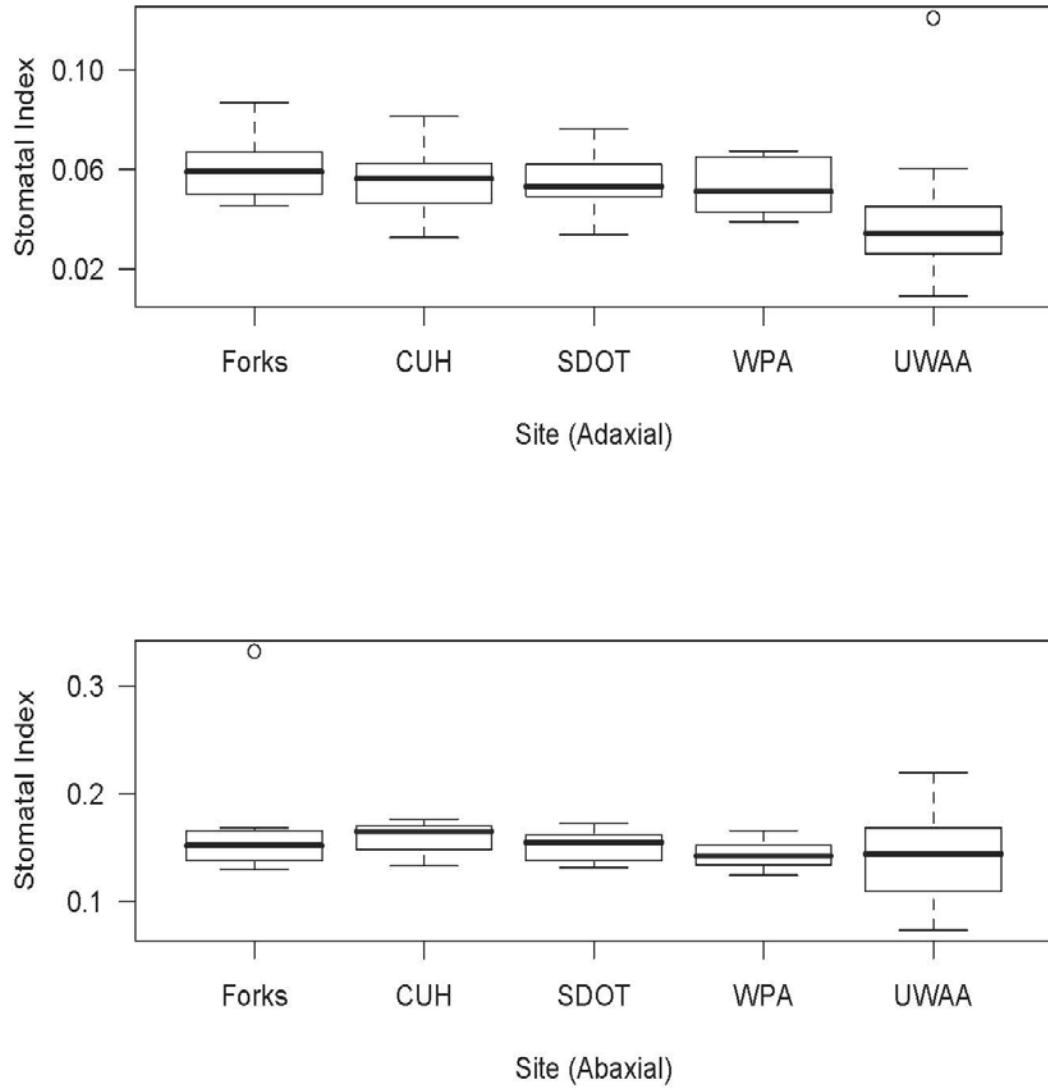
Fig. 32 Average stomatal index for both adaxial and abaxial surfaces of leaves for poplar hybrid plants grown at each site.

Surface	Site	SD±SE	(%)	SI±SE	(%)
	(n)	(mm^{-2})	±	(mm^{-2})	±
Adaxial	Forks (4)	82.67 ± 9.14		0.059 ± 0.006	
	CUH (4)	78.7 ± 11.06	-4.8	0.056 ± 0.006	-5.1
	SDOT (4)	77.38 ± 8.93	-6.4	0.055 ± 0.006	-6.8
	WPA (4)	76.72 ± 9.05	-7.2	0.053 ± 0.006	-10.2
	UWAA (4)	59.19 ± 16.24	-28.4	0.040 ± 0.015	-32.2
Abaxial	Forks (4)	191.14 ± 15.8		0.164 ± 0.027	
	CUH (4)	206.68 ± 15.24	7.52	0.159 ± 0.007	-3.04
	SDOT (4)	195.11 ± 10.42	2.05	0.151 ± 0.007	-7.93
	WPA (4)	188.82 ± 16.52	-1.2	0.143 ± 0.007	-12.8
	UWAA (4)	224.54 ± 33.8	14.9	0.142 ± 0.021	-13.4

3.4 Harvest results (A. thaliana)

In the field experiment the average total biomass (dry weight) per plant of the ecotype Col-0 was the highest for those grown at CUH with an average of 0.69 ± 0.196 (g). This average was significantly (0.005< p <0.01) higher than averages per plant grown in Forks which was 0.167 ± 0.074 (g). The Col-0 plants growing at SDOT had an average of 0.639 ± 0.152 (g) and were also significantly (0.01< p <0.02) higher than Col-0 plants growing at Forks. The average dry weight of Col-0 plants growing at WPA (0.118 ± 0.042 (g)/plant) and UWAA (0.052 ± 0.013 (g)/plant) were not significantly different than those grown in Forks (Fig. 24). Flower numbers for these plants revealed a similar trend where the average number of flowers for Col-0 plants grown at SDOT was 12.48 ± 4.81 while CUH plants averaged 11.0 ± 5.32 flowers per plant.

These averages were significantly (0.02< *p* <0.05) higher than that of plants

grown at Forks, which averaged 4.48 ± 1.87 flowers per plant. However,

although flower numbers per plant averaged 0.694 ± 0.327 at WPA and 2.44 ±

1.13 at UWAA, these were not significantly different than flower averages of

Col-0 plants grown in Forks (Fig. 25).
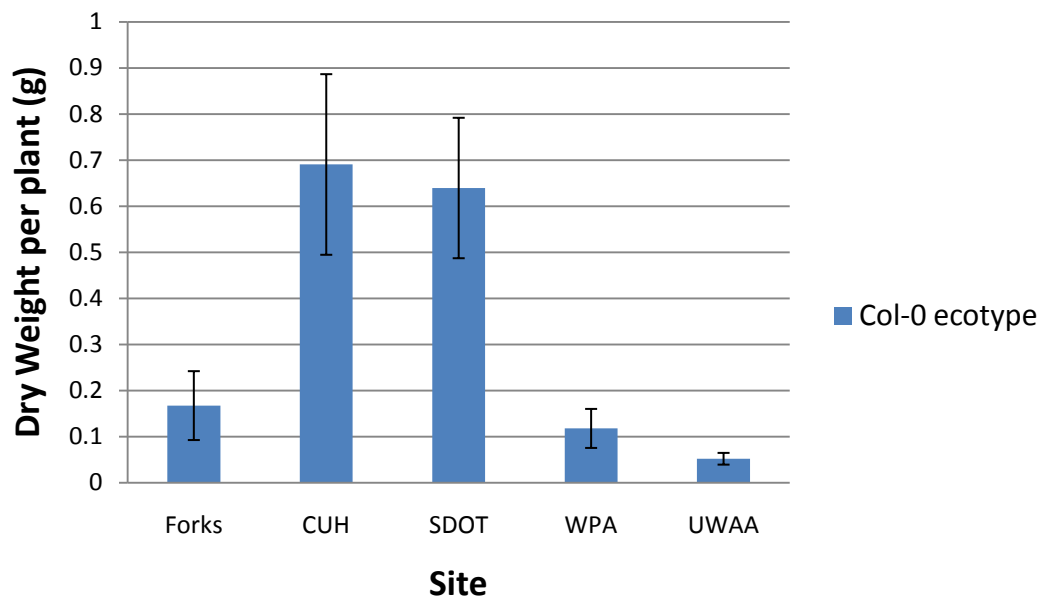
Fig. 24 Average total biomass for *A. thaliana* Col-0 ecotype plants grown at all sites in Seattle compared
to Forks. Error bars represent standard error.
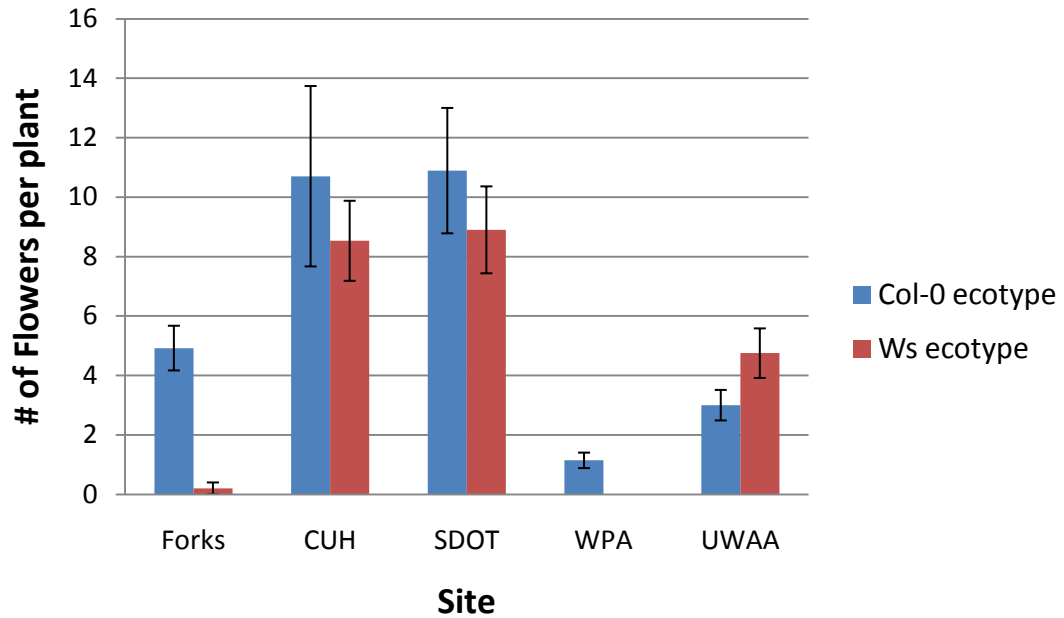
Fig. 25 Average number of flowers for *A. thaliana* plants grown at all sites in Seattle compared to Forks. Error bars represent standard error.

The average total biomass for Col-0 plants grown in [E]-CO_2 chambers was 0.64 ± 0.06 (g)/plant compared to those grown in [A]-CO_2 chambers, which averaged 0.32 ± 0.04 (g)/plant. These differences in dry weight were significant at a level between $0.002 < p < 0.005$ (Fig. 26). The average number of flowers per plant in [E]-CO_2 chambers was 10.8 ± 1.5, while those grown in [A]-CO_2 chambers averaged 8.3 ± 1.2 flowers per plant (Fig. 27). The average leaf area (cm^2) for the Col-0 plants grown in the [E]-CO_2 chambers was 175.5 ± 12.6 compared to the average leaf area of plants grown in the [A]-CO_2 chambers (104.3 ± 8.8). This difference in leaf area (cm^2) was significant at a level of ($0.001 < p < 0.002$).
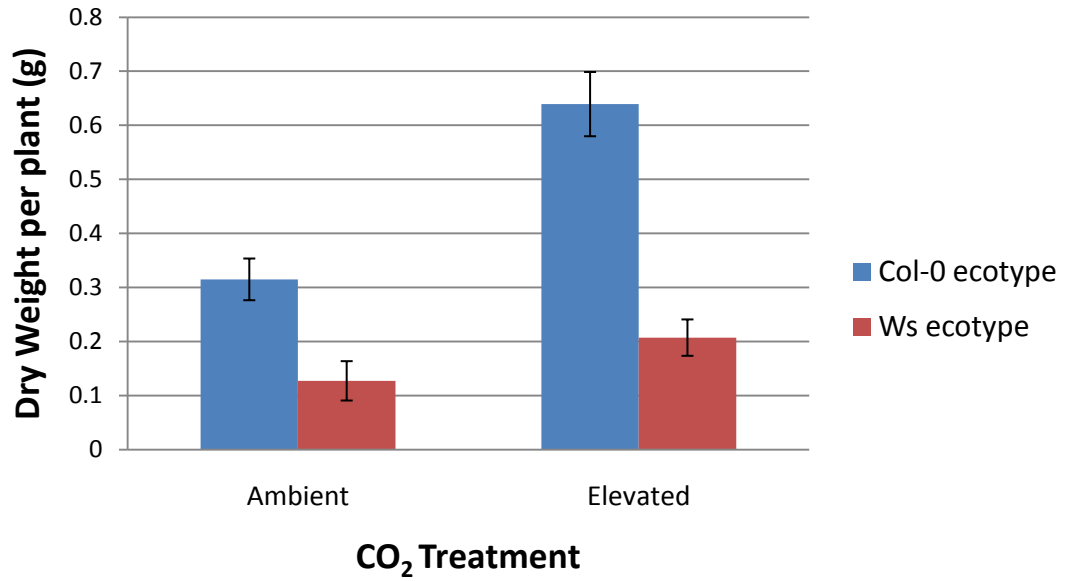
Fig. 26 Average total biomass for *A. thaliana* plants grown in closed-CO_2 chambers. Error bars represent standard error.
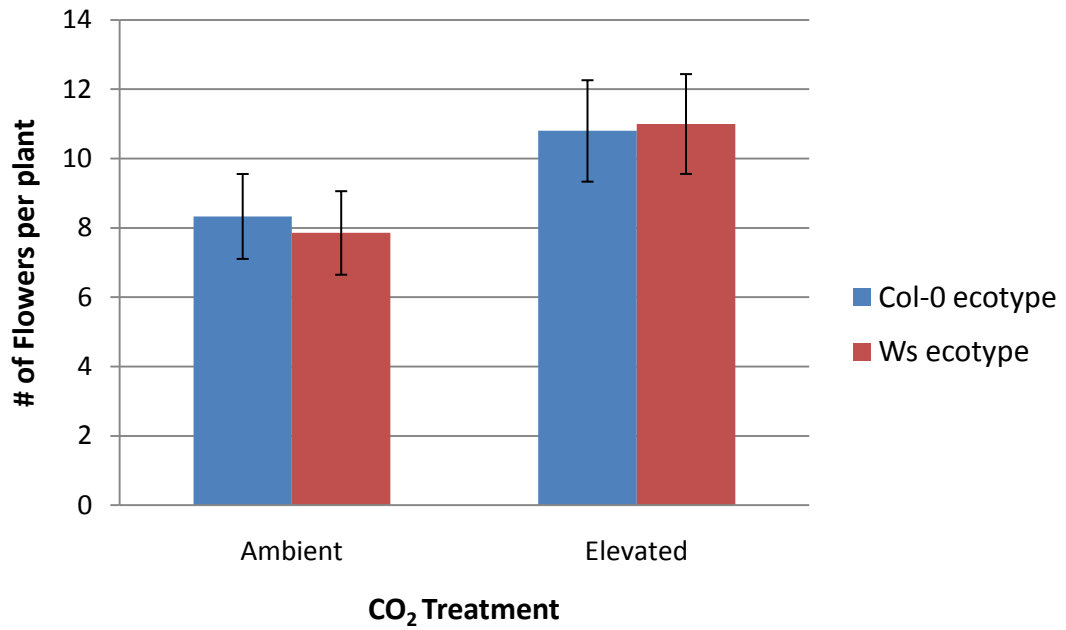
Fig. 27 Average number of flowers for *A. thaliana* plants grown in closed-CO_2 chambers. Error bars represent standard error.

A. thaliana plants (ecotype Ws) grown at SDOT had the greatest average biomass per plant at 0.665 ± 0.184 (g) followed by CUH with an average biomass of 0.569 ± 0.071 (g)/plant. These dry weights were significantly (0.02< *p* <0.05) higher than those grown in Forks, which averaged 0.120 ± 0.086 (g)/plant. Biomass averages at UWAA was 0.062 ± 0.015 (g)/plant, these values were not significantly different than average biomass of plants grown in Forks (Fig.28). This trend in biomass is repeated for average flower numbers per plant where SDOT Ws plants are the greatest at 13.46 ± 4.14 followed by CUH plants that averaged 9.66 ± 3.07. These averages are significantly higher for SDOT, (0.002< *p* <0.005), and CUH, (0.005< *p* <0.01) compared to Forks, which averaged 0.267 ± 0.267. Finally, UWAA averaged 3.15 ± 1.7 flowers per plant with no significant difference compared to flower numbers of plants grown in Forks (Fig. 25).
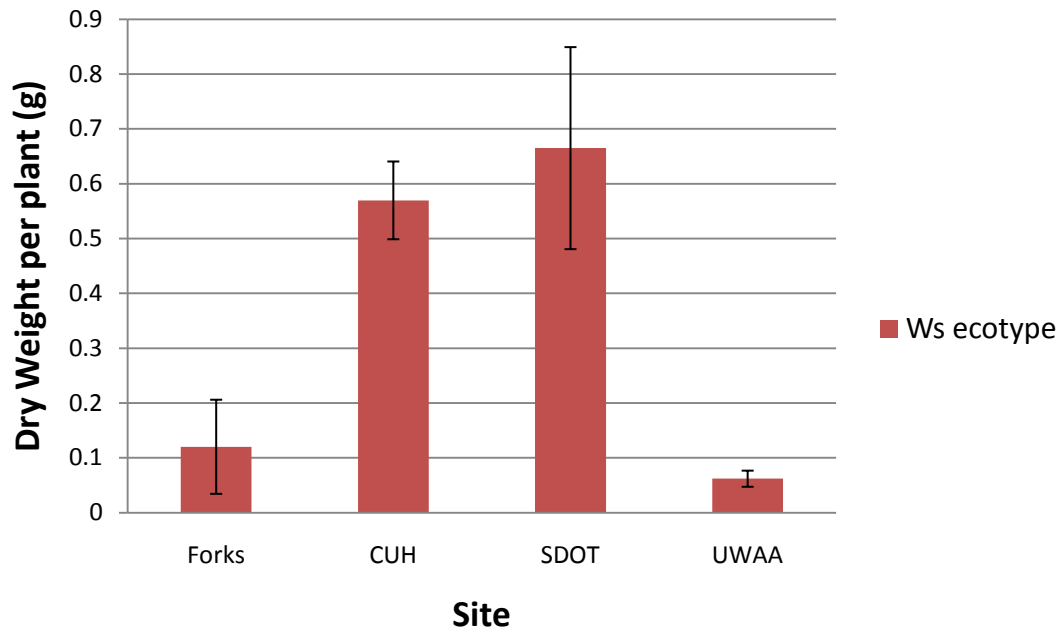
Fig. 28 Average total biomass for *A. thaliana* Ws ecotype plants grown in Seattle compared to Forks.
Error bars represent standard error.

For the Ws ecotype similar trends were observed between CO_2 treatments. The average biomass for Ws plants grown in [E]-CO_2 chambers was 0.21 ± 0.03 (g) while the average for plants grown in [A]-CO_2 chambers was 0.13 ± 0.04 (g). These average differences were significant at a level of (0.05 <*p*< 0.1) (Fig. 26). The average leaf area (cm^2) of Ws plants grown in [E]-CO_2 chambers was 54.9 ± 9.3 compared to an average leaf area (cm^2) of 26.2 ± 6.9 for plants grown in [A]-CO_2 chambers. These differences had a significance level of (0.02 <p<0 .05). Finally, the average number of flowers per plant for Ws plants grown in [E]-CO_2 chambers was 11 ± 1.4 while those grown in [A]-CO_2 chambers averaged 7.9 ± 1.2 flowers per plant with a significance level of (.05 <*p*< 0.1) (Fig. 27).

Ecotype	Site (n)	Biomass (g)/plant	% ±	p-value, α(2)	Flowers #/plant	% ±	p-value, α(2)
Col-0	Forks (5)	0.167±0.074			4.48 ± 1.87		
	CUH (4)	0.69±0.196	75.8	0.005<p<0.01	11.0 ± 5.32	59.3	0.02<p<0.05
	SDOT (5)	0.639 ± 0.152	73.9	0.01<p<0.02	12.48 ± 4.81	64.1	0.02<p<0.05
	WPA (3)	0.118 ± 0.042	-29.3	0.5<p	0.694 ± 0.327	-84.5	0.5<p
	UWAA (4)	0.052 ± 0.013	-68.9	0.2<p<0.5	2.44 ± 1.13	45.5	0.2<p<0.5
Ws	Forks (2)	0.120 ± 0.086			0.267 ± 0.267		
	CUH (4)	0.569 ± 0.071	78.9	0.02<p<0.05	9.66 ± 3.07	97.2	0.005<p<0.01
	SDOT (4)	0.665 ± 0.184	81.9	0.02<p<0.05	13.46 ± 4.14	98.2	0.002<p<0.005
	UWAA (3)	0.062 ± 0.015	-48.3	0.5<p	3.15 ± 1.7	91.5	0.05<p<0.1

Ecotype	CO_2 (n)	DW ±	%	p-value, α(2)	Flower #/plant	%	p-value	Leaf Area ±	%	p-value, α(2)
Col-0	[A]-CO_2 (6)	0.32 ± 0.04			8.3 ± 1.2			104.3 ± 8.8		
	[E]-CO_2 (5)	0.64 ± 0.06	50	0.002<p<0.005	10.8 ± 1.5	23.1	0.2<p<0.5	175.5 ± 12.6	40.6	0.001<p<0.002
Ws	[A]-CO_2 (7)	0.13 ± 0.04			7.9 ± 1.2			26.2 ± 6.9		
	[E]-CO_2 (8)	0.21 ± 0.03	38.1	0.05<p<0.1	11 ± 1.4	28.2	.05<p<0.1	54.9 ± 9.3	52.3	0.02<p<0.05

3.5 Harvest Results (Populus deltoides x trichocarpa)

Total biomass (g), and total leaf area (cm^2) of hybrid poplar plants grown at the CUH, SDOT, WPA, and Forks sites had similar averages. Those grown at CUH had the highest average dry weight of 109.7 ± 7.07 (g)/plant while those grown in Forks averaged 91.7 ± 18.7 (g). The average dry weight from UWAA was the lowest at 26.2 ± 6.6 (g)/plant which was significantly ($p<0.005$) lower than biomass averages of poplar hybrids grown in Forks (Fig. 29).
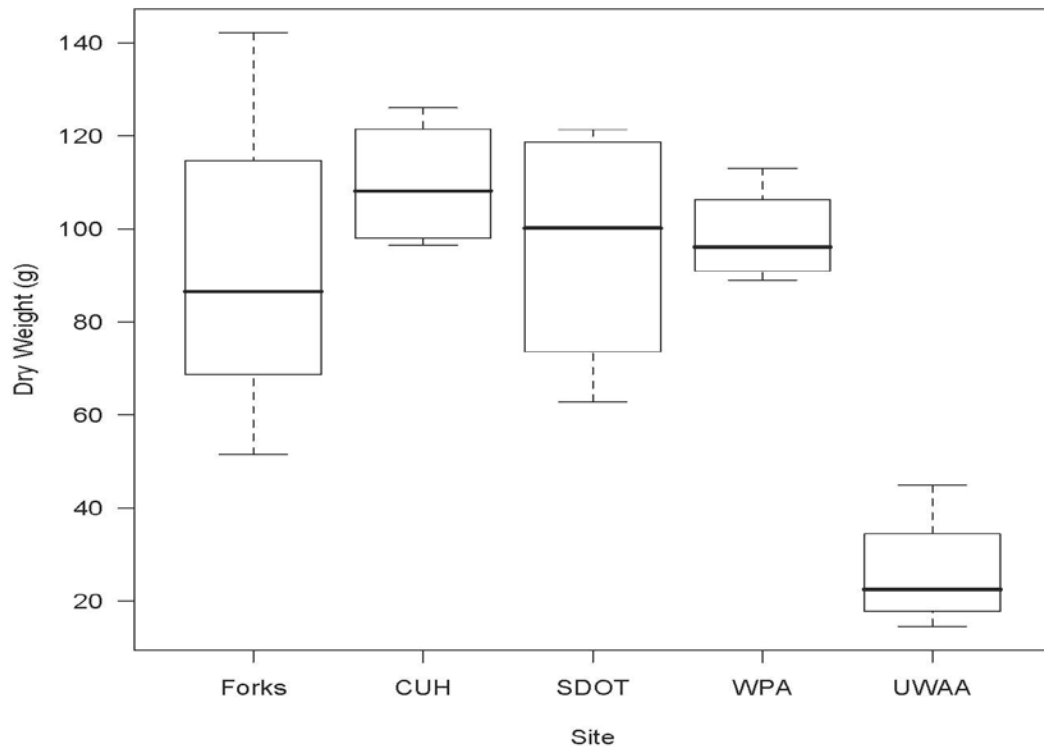
Fig. 29 Average total biomass per plant for poplar hybrids grown at each site from July- September, 2008.

Similarly, leaf area (cm^2) did not differ much between plants grown at the CUH, SDOT, WPA and Forks. The highest average was 4814.4 ± 407.2 (mm^{-2}) for plants grown at the WPA in Seattle while the lowest average was 3388.9 ± 59 (mm^{-2}) per plant grown in Forks. Again, plants grown at the UWAA site had significantly (p<0.005) lower average leaf area (cm^2) than plants grown at the other sites (Fig. 30).
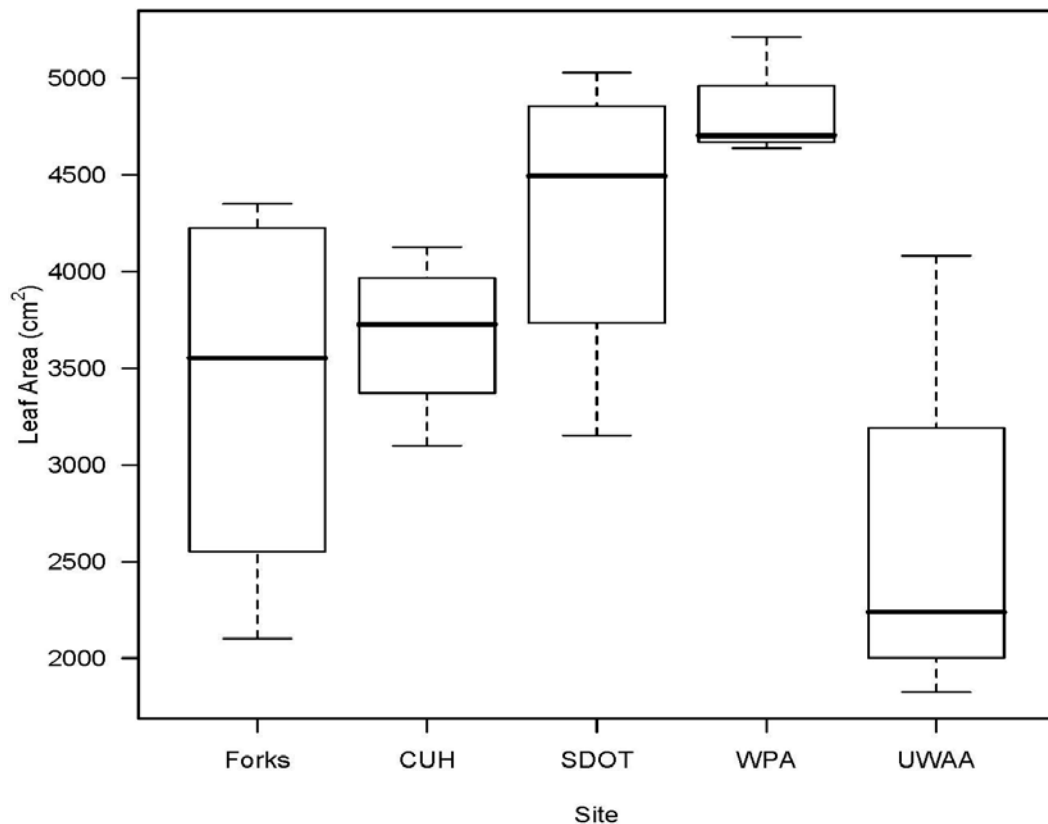
Fig. 30 Average total leaf area per plant for poplar hybrids grown at each site from July- September, 2008.

Site (n)	DW	% ±	Leaf Area	% ±
Forks (4)	797.13 ± 18.73		3388.98 ± 519.38	
CUH (4)	690.83 ± 7.07	-13.4	3669.95 ± 214.91	7.7
SDOT (4)	907.16 ± 13.78	12.1	4292.62 ± 407.48	21.1
WPA (4)	844.84 ± 5.28	5.5	4814.35 ± 133.87	29.6
UWAA (4)	729.03 ± 6.56	-8.5	2595.62 ± 505.6	-23.4

4. Discussion

The dual goals of this study were met by documenting the differences in environmental variables of interest (namely CO_2 and air temperature) between sites in Seattle and Forks and by measuring plant growth (biomass, flowers etc.) and anatomy (stomatal numbers) in response to the environmental variables they were exposed to.

4.1 Environmental variables

Seattle, located on the eastern shore of the Puget Sound has a constant airflow from the Pacific Ocean, and aside from rare temperature inversions, typically has well-mixed clean air (pscleanair, 2009). The aforementioned study reveals that the concentrated anthropogenic fossil fuel emissions have led to elevated CO_2 levels within the city. Even with the region's well-mixed airflow, CO_2 concentrations at CUH and WPA in Seattle averaged 8.1 and 5.6% higher than those observed at the rural site in Forks. The urban centers of Phoenix and Baltimore averaged 38-43% and 16-31% higher CO_2 concentrations, respectively, than their surrounding rural areas (Idso *et al.*, 2001 and George *et al.*, 2007). Our observed CO_2 concentrations

within Seattle represent a similar, although less pronounced "CO_2 dome". In addition, the increase in average air temperature (day and nighttime) at both CUH and WPA sites in Seattle compared to Forks resemble trends in cities that have documented urban heat islands (Oke and Maxwell, 1975). Relative humidity remained above 50% at all locations and was similar between sites indicating it did not have a strong effect on the growth of plants (Aphalo and Jarvis, 1991). The effects of differing light levels at each site will be discussed in the following section.

4.2 Stomatal numbers

Stomatal density (SD), epidermal density (ED) and stomatal index (SI) are numerical measurements of plant anatomy that can vary between plants of the same and different species over generations and within a lifetime.

Woodward (1987) describes how many plant species respond to increasing levels of CO_2 by decreasing their SD. However, Reid (2003) reviews literature that is contradictory to the common belief that increased levels of CO_2 lead to a decrease in SD. Data from 15 different plant species studied over four years shows no stomatal response to increased levels of CO_2 ranging between 200 and 550 µmol mol^{-1} of CO_2. The possible explanations as to why no response to elevated levels of CO_2 was detected are numerous and varied. For example the particular species sampled may not be responsive to elevated CO_2 concentrations; i.e. not all responded in the

Woodward, 1987 study. In addition some research suggests that they may

have reached their "CO_2 ceiling"- a phenomenon termed to describe the point

where SD and SI no longer respond to levels of CO_2 higher than current

ambient levels (Roth-nebelsick, 2005).

Nonetheless, a reduction in SD is a common response of plants to

elevated CO_2 concentrations and has been documented in many experiments,

particularly in controlled environments (Miyazawa *et al.*, 2006). Royer

describes in a 2001 review, when variables such as light, relative humidity and

CO_2 differ (as they may in field settings) then SI is a more illustrative

representation of stomatal numbers per plant. As a ratio, SI is independent of

cell size, which can alter SD and ED rather than the actual number of pores

and cells per plant. This is due to the fact that in response to increased CO_2

the leaf area (cm^2) and epidermal cell sizes of plants can increase, leading to

a decrease in SD, not a decrease in stomatal numbers per leaf (Chen et al.,

2001). For these reasons we look at SI in addition to SD and ED in the

comparisons of stomatal numbers in the field setting where environmental

variables could not be controlled.

Table 2 synthesizes the information regarding SD and ED for the *A.*

thaliana species and overall there was a significant decrease for both of these

variables at all sites in Seattle except WPA compared with Forks for the Col-0

ecotype. When SI is compared between sites significance remains only for the abaxial surface of plants from CUH and both surfaces of UWAA plants (Table 3). The stomatal numbers of *A. thaliana* ecotype Col-0 tend to decrease when grown under shaded and low light conditions (Lake et al., 2001). This suggests that the significantly lower light levels at UWAA were responsible for the relatively large decrease in stomatal numbers observed for plants grown there. However, the canopy was relatively open and light levels were similar at CUH and Forks (Table 1 & Fig. 9) indicating that CO_2 was indeed the environmental factor driving the response in a reduction of stomatal numbers observed for these plants. Had light been more influential in decreasing stomatal numbers at the other Seattle sites it is likely that a more significant decrease in SD for plants grown at SDOT and WPA would have been observed, as their percent canopy cover is lower than the CUH. Instead, average SD of Col-0 plants from SDOT and WPA is higher than CUH plants (Table 2).

Furthermore, results from the chamber portion of this experiment provide additional evidence that CO_2 was the driving environmental factor that led to a decrease in stomatal numbers for Col-0 plants grown in the field. The SD was significantly lower for the abaxial surface of these plants grown in [E]-CO_2 chambers while ED and SI did not differ (Table 4). CO_2 concentration was the only treatment in the chambers and ED did not change (indicating cell size was not altered) making CO_2 responsible for this observed decrease in

SD. These results that coincide between the field and chamber experiments

confirm that the observed reduction in stomatal numbers for the *A. thaliana*

ecotype Col-0 is in response to the elevated levels of CO_2. A reduction in

plant stomatal numbers in response to CO_2 has been shown in previous

chamber studies such as: Lake *et al.*, 2001, Woodward *et al.*, 2002, historical

herbarium samples (Woodward *et al.*, 1987) and fossilized plant material

(Beerling and Royer, 2002). However, we believe that field data examining

whether urban CO_2 concentrations influence stomatal numbers has not been

collected and documented until now.

In addition to documenting a stomatal response over a range of species

exposed to varying levels of CO_2, Woodward and colleagues show that within

one species there can be different stomatal responses to CO_2 depending on

the ecotype (Woodward *et al.*, 2002). In the present study we found this to be

true as the SD and ED for the *A. thaliana* (ecotype Ws) were not significantly

different among plants grown at any of the sites (Table 2). The SI of Ws

plants from UWAA was significantly lower compared with that of Forks (Table

3) and as before we relate this response to the low light levels observed at

UWAA. It was observed for the same ecotype grown in the [E]-CO2 chambers

that SD and ED decreased significantly on the abaxial surfaces of these

leaves while the SI actually increased slightly on the abaxial surface and

significantly on the adaxial surface (Table 4). It could be that sample size was

not large enough to draw more significant and clear conclusions but certainly

the range of intra-specific variability can be seen here.   One suggestion as to why the Ws plants responded differently to CO_2 in the field and chamber settings relates to the large difference in CO_2 concentrations in the two studies. While values averaged around 740 ppm in the [E]-CO2 chambers, they only reached an average high of 427 ppm at the sites in Seattle thus indicating the possibility that, average CO_2 concentrations necessary to induce such a response had not been reached in the field.

Poplar hybrids have also been shown to decrease their stomatal numbers in response to elevated CO_2 concentrations in controlled environments (Miyazawa *et al.*, 2006). In their 2006 study, Miyazawa and colleagues found fewer stomatal numbers for the upper-most expanded leaves of poplar hybrids compared to the mature leaves of the same plant that were exposed to elevated concentrations of CO_2 averaging 780 ppm. This led them to conclude that hormonal signals sent by mature leaves to new growth determine the stomatal numbers of new leaves depending on the environment around the mature leaves. It was for these reasons we hypothesized that stomatal numbers of leaves that developed at the sites in Seattle, where CO_2 concentrations were higher, would be lower compared to the mature leaves of the same plants that developed in the greenhouse where they were propagated. We failed to observe significant differences in stomatal numbers according to this hypothesis and there are a couple of suggestions as to why this was the case. First, CO_2 concentrations in the greenhouse (where the

poplar hybrids were propagated) may have been relatively high as some greenhouse are due to plant respiration at night. Second, although average SD and SI were lower at sites in Seattle (Figs. 25 & 26) it is possible that the limited sample size of plants growing at each site was not enough to detect a significant difference in these stomatal numbers.

4.3 Plant growth

Increased CO_2 concentrations and air temperature in the city positively affect plant biomass and pollen accumulation rates as well as lead to an increase in the number of growing-degree-days relative to surrounding rural areas (Ziska *et al.*, 2003, 2004, 2007). The significantly higher biomass of *A. thaliana* plants (both ecotypes) grown in Seattle compared with Forks and those grown in the chamber experiment seem to confirm the effects of CO_2 enrichment on biomass that Ziska *et al.* (2003) have documented. It is interesting to note here that total plant biomass averages were significantly higher at SDOT and CUH compared with Forks and WPA. Plants grown at WPA did not exhibit growth responses similar to SDOT and CUH even though light levels were similar to SDOT and CO_2 concentrations were higher than Forks. This suggests other environmental variables may be playing a role in plant growth in urban locations, one that is present at SDOT and CUH and not at WPA.

Woodward *et al.* (2002) discusses the positive effects of elevated CO_2

(700ppm) on flower numbers for the *A. thaliana* ecotype Col-0 but not for the

ecotype Ws. In the present study flower numbers for plants grown in the field

setting followed the same trends as biomass where SDOT and CUH were

significantly higher on average compared with Forks for both ecotypes. This

response is seen only for the Ws ecotype plants grown in [E]-CO_2 chambers,

which is opposite from results in the Woodward *et al.*, 2002 study suggesting a

varied response. The timing of plant retrieval in the field setting may not have

been conducive to estimating the flower numbers of plants and comparing

them between sites. As a remote location, Forks was difficult to access on a

regular basis making it challenging to determine exact dates of flowering per

plant hence the possibility that flowers may have already dropped or not yet

developed depending on the ecotype and timing of experiment. These

scenarios lead to the conclusion that flower numbers in this study are not

indicative of a significant growth response to CO_2 in both field and chamber

settings. Finally, leaf area for both ecotypes grown in [E]-CO2 chambers was

significantly higher than those grown in [A]-CO_2 chambers indicating a positive

response to CO_2 in this setting; however, due to sampling methods for plants

grown in the field this comparison could not be made.

The plant biomass and leaf area averages of the poplar hybrids

followed a similar trend as the stomatal numbers of these plants. While on

average those grown in Seattle had a greater dry weight and total leaf area,

we failed to observe any significant differences among any of the sites with an exception for UWAA where the low light levels due to surrounding buildings and trees was likely the cause for growth reduction.

5. Conclusions

Through this research it has been shown that the climatic conditions (specifically, CO_2 concentrations and air temperature) in the city of Seattle are higher than the rural location of Forks and indicative of expected future global climate trends. In addition, *A. thaliana* plants showed significant changes in their biomass accumulation, stomatal numbers, flower numbers and leaf area in response to the urban climate that has never been documented before. This twofold response substantiates claims made by Ziska *et al.* (2003) that the urban environment can be used as a harbinger and living laboratory in which we can study plant response to climate change.

5.1 Recommendations

As the threat of climate change and greenhouse gases in the atmosphere is further reported, accumulating knowledge about their effects is critical. By setting up weather stations in many contemporary cities more information, including short- and long-term variations in climatic variables as well as the range (ex: lowest to highest CO_2) of these variables among cities.

These climatic variables of urban centers will be valuable in assessing and understanding plant response to climate change. As we saw a range of plant responses between the two different species (hybrid poplar and *A. thaliana*) and within one species in this study there remains more information to be determined, such as which species and to what extent are they affected by elevated levels of CO_2 and air temperature. It is recommended to increase the minimum number of hybrid poplar plants to 11 at each site (in order to obtain a 95 percent chance of detecting a true difference between the means) for future studies.

For the *A. thaliana* ecotypes it is recommended that flower numbers are monitored on a more constant basis in order to determine more accurate data for this variable in the urban setting. In addition, growing the *A. thaliana* plants over a range of densities in the urban setting may provide insight into what was responsible for the plant biomass accumulation differences found among sites in Seattle. Furthermore, by testing for the carbon isotopic composition ($\delta^{13}C$) of these plants, information regarding the amount of carbon they are exposed to from fossil-fuel emitting sources can be determined by directly connecting anthropogenic sources of emissions to the plant anatomical responses (Kelome *et al.*, 2006).

Collecting additional field information regarding soil moisture and plant-water relations as well as broadening the range of plants sampled in the urban setting will strengthen the knowledge on the anatomical and growth responses

that we've detected. By sampling leaves of common street trees like the tulip poplar and red oak, it can be determined whether these plants are responding anatomically as we observed the model research *A. thaliana* plants are. If they in fact show a response, then it would be appropriate to bring these findings to a greater scale and apply modeling techniques in order to evaluate how these large scale changes can affect plant-soil water relations throughout the urban settings. Gedney *et al.* (2006) describes a modeling scenario where increased CO_2 can lead to a decrease in leaf conductance and then when scaled up to canopy and continental levels the result is increased runoff worldwide. This type of information is vital in revealing the scope of large scale plant response to climate change and how major processes such as the hydrologic cycle could be affected.

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Appendix A: List of Acronyms

CUH: Center for Urban Horticulture

ED: Epidermal Denisty

SD: Stomatal Density

SE: Standard Error

SI: Stomatal Index

SDOT: Seattle Department of Transportation

UWAA: University of Washington Alumni Association

WPA: Washington Park Arboretum