5-2003

Determining the Factors That Control Respiration and Carbon Use Efficiency in Crop Plants

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DETERMINING THE FACTORS THAT CONTROL RESPIRATION

AND CARBON USE EFFICIENCY IN CROP PLANTS

by

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A dissertation submitted in partial fulfillment
of the requirement for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Science
(Crop Physiology)

UTAH STATE UNIVERSITY
Logan, Utah

2003

ABSTRACT

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and Carbon Use Efficiency in Crop Plants

by

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In the literature on plant respiration, there are two viewpoints concerning the source of respiratory control: supply (photosynthate availability) or demand (temperature dependent) limitations. While different studies indicate the primary dependency for respiration is either the supply or demand side, the two paradigms cannot both be true. The relative importance of each paradigm may depend on a number of factors including period of time during which respiration is measured, phase of plant development, environmental conditions, and species.

Studies were performed using continuous CO_2 gas-exchange instrumentation to monitor short- and long-term changes in whole canopies of lettuce, tomato, soybean, and rice in response to changes in light and temperature during vegetative growth. Respiration in all crops was less sensitive to temperature than previously reported. This is likely due to large amounts of temperature-insensitive growth respiration as a fraction

of total respiration during early growth. Carbon use efficiency (CUE) decreased with warm night temperatures, but the change was too small to decrease the final dry mass or carbon gain after night temperatures decreased. Canopies with constant day/night temperature had the same CUE, in elevated CO_2 (1,200 μmol mol^{-1}), regardless of temperature. In ambient CO_2 (400 μmol mol^{-1}), CUE decreased significantly when temperatures were above 32C.

Applying shade initially decreased CUE because of low photosynthesis and high respiration. After about 12 days, canopies acclimated, based on recovery of CUE. Different species acclimated to shade to different extents, but no interaction was evident between light and shade stress. These data were used to predict changes in photosynthesis, respiration, and carbon use efficiency given light, temperature, and CO_2 concentrations.

(180 pages)

DEDICATION

To Susan and Henry.

ACKNOWLEDGMENTS

I want to thank my major professor, Dr. Bruce Bugbee, for his patience and enthusiasm, and for allowing me the freedom to get my hands in as much as I could. I want to thank Dr. Paul Johnson for his behind-the-scenes support, Dr. Ray Wheeler for discussions at meetings about my project, wherever we were, Dr. Keith Mott for helping me to clarify why I'm doing this in the first place, Dr. James Haefner for opening up the world of models to me, and finally, Dr. Doug Johnson for helping me stay the course in research and course work.

I want to also thank Dr. Marc van Iersel for new insights into old problems, helpful reviews, and for all additional collaborations. I've had the opportunity to work with the great people in the crop physiology laboratory, and have had the pleasure of working side-by-side with a talented and motivated undergraduate student, Justin Robinson. Thank you for all your help.

Jonathan M. Frantz

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CHAPTER 1

INTRODUCTION

Plant respiration has been studied far less than animal respiration. Studies on

human and mammalian respiration assist in diagnosing disease, understanding nutritional

requirements, and determining some behavior patterns. Just as an understanding of the

factors that determine respiration rates in animals help in understanding many aspects of

animal physiology, understanding the factors that determine respiration in plants may

help predict carbon balance in plants, assist in determining stress affects, and help predict

yield. Since more is known about respiration in animal systems, plant research could

benefit by understanding the factors known to affect respiration rates in animals and

gaining familiarity with some of the techniques used to study these factors. Insight could

be gained on how plants could be studied most effectively.

While the literature discussing control of animal respiration focuses on the animal-

environment interface, some general characteristics help to determine the oxygen demand

for a given animal. These characteristics are 1) external oxygen supply, 2) species, 3)

activity level of the animal, 4) size of animal, and 5) temperature of the environment

(Dejours, 1981). Each of these are analogous to characteristics that determine the

respiration rate of crop plants.

Animals inhabit a wide range of environments. Occasionally, animals encounter, or

in some instances desire, hypoxic or anoxic conditions, which can limit the availability of

oxygen for respiring cells. When this occurs, animals either seek more aerobic

environments or adapt their metabolism to produce some energy anaerobically. In crop

plants, the external oxygen supply is rarely limiting. In flooded soils, rootzones can become hypoxic and so must cope with either obtaining oxygen from the shoot via aerenchyma, or activate enzymes that can allow respiration to occur anaerobically and produce smaller amounts of ATP.

Species and activity level can have a large affect determining the metabolic rate in animals (Cameron, 1989). For a given size of animal, different species have different amounts of muscle tissue, skeletal mass, and diet as well as different capabilities for activity. All of these will determine their oxygen needs through basic "maintenance" requirements to sustain life and requirements to begin and sustain different levels of activity. Similarly, different plant species of the same size have different metabolic requirements, which correspond to their growth rates (Lambers et al., 1998). For example, a corn plant will have higher respiration rates than a native grass species of the desert southwest. The reason for this is that corn has been bred for fast *growth* across a finite growing season in a virtually stress-free environment, while the grass is adapted for *survival* in a hot, dry environment.

There is a strong relationship between the size of an animal and its total respiration rate (Dejours, 1981). This makes sense because the more muscle mass, for example, the more oxygen the animal would need to allow the muscle to work. In plants, there is a similar relationship (Enquist et al., 1998). The more leaf, root, and meristematic tissue, the larger the oxygen requirement for maintenance and growth. Generally, long-term productivity of a group of plants can be predicted based on some measure of size (height, mass, stem diameter) because of the relationship between productivity and light

interception (Enquist et al., 1999). On shorter time scales (vegetative phase of growth), the relationship may not hold (Niklas and Enquist, 2001).

Finally, temperature strongly influences the respiration rate in animals, although this relationship depends on the species (Cameron, 1989). In ectotherms (also known as "cold-blooded animals"), increasing temperature raises the metabolic rate, with a Q_{10} of approximately 2. The increase in metabolism is associated with increased protein turnover and enzymatic reactions, but also usually coincides with an increase in activity for the animal. Therefore, it is difficult to separate the behavioral response to a rise in temperature from a biochemical response. In homeotherms (also known as "warm-blooded animals"), the metabolic response to temperature is very different. Any deviation from the core body temperature will increase the metabolic rate. This is believed to be caused by an increase in heat generation through respiration during a decrease in temperature and an increase in cooling (more circulation, sweating, etc.) when the temperature increases.

Plants have a temperature response that is similar to ectotherms (Q_{10} of about 2). These studies in both plants and animals were conducted in short-term experiments lasting a few hours across a wide range of temperatures, the importance of which will be discussed later.

The literature about respiration control in animals focuses on aspects that are primarily external to the animals. Respiration control for animals means maintaining a homeostatic oxygen concentration within the cells and supply to the cells (Dejours, 1981). That is, control of respiration is when animals perceive the environment and then

either reflexively modify their association with that environment or use their locomotive abilities to move to a more suitable environment to maintain their oxygen supply. Plants, unlike animals, are unable to actively seek better environments in which to grow. Consequently, control of respiration has been thought to be maily dependent on the surrounding environment.

Most studies investigating oxygen consumption in animals are conducted by measuring the amount of O_2 that is dissolved in blood, blood pressure, and/or surgically investigating oxygen status on live organisms. In a few cases, gas exchange measurements can be made by recording the oxygen concentration of each breath for different activities. While each of these techniques for measuring the gas-exchange capabilities of resting animals is straightforward, difficulties arise when active animals need to be studied *in situ* (i.e., diving ducks, hibernating mammals, flying birds, or migrating fish). Plant respiration has been studied in far less detail than animal respiration. This is partly because it is difficult to study plant respiration during a 24-h period because of photosynthesis in the light. The rate of O_2 consumption or CO_2 generation in live tissue in the light cannot be measured because photosynthesis is giving off oxygen and taking up CO_2 at the same time. A further complication is photorespiration whereby ribulose-1,5-bisphosphate is oxygenated to form one phosphoglyceric acid (PGA, C3) and one phosphoglycolate (C2) molecule rather than 2 PGA molecules during carboxylation. Because of these technical problems, the control of respiration is poorly understood in plants.

In the literature on plant respiration, there are two viewpoints concerning the source

of respiratory control: supply (photosynthate availability) or demand (temperature dependent) limitations (Amthor, 1989). While different studies indicate the primary dependency for respiration is either the supply or demand side, the two paradigms cannot both be true. The relative importance of each paradigm may depend on a number of factors including period of time in which respiration is measured, phase of plant development, environmental conditions, and species. These two viewpoints and the methods for calculating carbon use will now be discussed in more detail.

Calculating Carbon Use Efficiency

Central to my research is the determination of carbon use efficiency (CUE, also known as growth efficiency or respiration efficiency). Using gas-exchange data collected during a 24-h period, CUE will be quantified according to Figure 1-1 and the accompanying equations. These calculations assume that respiration rates are identical in the day and night (Dewar et al., 1998). It is difficult to get estimates of day-time respiration rates, and these estimates depend on the method used. An average value obtained from two methods indicated that day-time respiration rates in leaves are as much as 55% lower than night-time respiration rates (Villar et al., 1994). Furthermore, cyanide-resistant respiration of photosynthetic cells in the light suggests that the mitochondrial electron transport chain does not function at all or at the same level as in the dark, which would decrease dark respiration rates in the light (Azcón-Bieto and Osmond, 1983). Baker et al. (1972) estimated day-time respiration by temporarily placing leaves in darkened chambers during the light period, and daytime rates were

reported to have exceeded night-time rates by 20 to 25%. Meristematic cells and roots, however, increase their respiration rates in the light, which could offset a decrease in dark respiration in photosynthetic cells in the light (Robson and Parsons, 1981; Monje and Bugbee, 1996). Even if the day and night respiration rates are not identical, CUE changes by only 10% if daytime respiration rates are 50% different from night time respiration rates (Monje and Bugbee, 1998). Therefore, the assumption that daytime respiration rates are the same as night-time respiration rates is fairly robust, and from this point, this assumption will drive the calculation of CUE as written in Figure 1-1.

Supply

Numerous studies indicate that respiration rate is highly correlated with carbon substrate availability, which is determined by photosynthetic rate (McCree, 1974; Coggeshall and Hodges, 1980; Moser et al., 1982; Azcon-Beito et al., 1983; Azcón-Bieto and Osmond, 1983; Stitt et al., 1990; Krapp et al., 1991; Geiger et al., 1998; Roberntz and Stockfors, 1998). Increasing photosynthetic rate increases the substrate concentrations for a whole plant (Stitt et al., 1990; Smart et al., 1994; Roberntz and Stockfors, 1998). This substrate is correlated with increased amount and activity of glycolytic enzymes, the first steps in respiration (Stitt et al., 1990; Krapp et al., 1991; Geiger et al., 1998).

Respiration rate was positively correlated with the nonstructural carbohydrate concentration in leaves of several different species (Azcón-Bieto and Osmond, 1983; Azcón-Bieto et al., 1983). To obtain an estimate of the nonstructural carbohydrate supply of these leaves, samples of similar sized and aged leaves were destructively harvested and

carbohydrates were extracted. Interestingly, pea leaves (*Pisum sativum*) behaved slightly different than wheat (*Triticum aestivum* L.) and spinach (*Spinacia oleracea*) leaves. Pea carbohydrate concentration did not change significantly during the course of 5 to 7 hours of darkness while that of wheat and spinach decreased substantially. This suggested that pea leaves were strong "sinks" for carbohydrate compared to other species or that they rapidly mobilized their own starch reserves. Coinciding with the constant carbohydrate concentration in pea leaves was an approximately constant respiration rate throughout the night. Exogenous applications of sugar to plant tissue also can stimulate respiration, as measured by leaf-segment gas-exchange (Krapp et al., 1991; Hill and Rees, 1995). Unfortunately, individual leaf segments or organs of a plant are not representative of whole plant respiration (McCree and Amthor, 1982). Therefore, evidence obtained from plant parts when used to describe whole-plant and community dependencies for respiration control must be accepted with caution.

Daytime photosynthetic rates of leaf segments or whole leaves have been correlated with respiration rates in a variety of species (Azcón-Bieto and Osmond, 1983; Azcón-Bieto et al., 1983; Thomas and Griffen, 1994; Baxter et al., 1995; Roberntz and Stockfors, 1998). A leaf or leaf segment was placed within a darkened cuvette and O_2 consumption and/or CO_2 generation were measured for short (minutes to hours) periods of time. The rate of respiration was strongly correlated to the rate of prior CO_2 assimilation in the light period.

Only a few studies have attempted to show a correlation between carbohydrate supply and respiratory control using whole plants (McCree and Troughton, 1966; Wilson

et al., 1978; McCree, 1982). In these studies, whole-plant dark respiration rates were positively correlated to daytime photosynthetic rates. Plants were placed in the dark for brief periods (up to 30 min) in the daytime, and these respiration rates were positively correlated to photosynthetic rates immediately previous to the dark treatment. When plants were shaded, causing a reduction in photosynthetic rates as well as carbohydrate content, nighttime respiration rates decreased, which resulted in a constant ratio of daily photosynthesis to respiration (McCree and Troughton, 1966). Nighttime respiration rates were higher in longer photoperiods and lower in shorter photoperiods, which resulted in a constant ratio of daily photosynthesis to respiration. The longest night-time respiration rate measurement in these studies was 6 to 15 hours, depending on the photoperiod.

Demand

Respiration rates typically increase with temperature (McCree and Amthor, 1982; Labate and Leegood, 1989; Roberts et al., 1992; Wheeler et al., 1993; Smith and Wu, 1994; Maier et al., 1998) until the temperature reaches about 35 to 40C. Respiration rates generally plateau at the next 5 to 10C, and then fall (Roberts et al., 1992). These rates coincide with changes in protein turnover rates and rates of enzymatic reactions (McCree and Amthor, 1982; Roberts et al., 1992). During the increase phase, respiration has a Q_{10} of between 1.7-2.5, which means that respiration approximately doubles for every 10C increase in temperature (McCree and Amthor, 1982; Johnson and Thornley, 1985). To experimentally determine this value, temperature is changed during the course of a few hours during a dark period, usually for single plants or leaves, and respiration rates are

measured across a range of temperatures (Crawford and Huxter, 1977; Alexander et al., 1995). The extent of temperature dependency and the range of temperatures at which respiration is affected changes slightly for different crops as does the value of Q_{10}. These techniques clearly indicate that temperature influences respiration rates. The short-term nature of the studies, however, do not provide any indication of how plant tissue might adapt to a particular temperature and the resulting increase or decrease the rates of physiological processes.

There have been several attempts at correlating maintenance respiration requirement to N content or protein content (Ryan et al., 1996; Dewar, 1996). It is believed that N status is a reflection of protein amount and therefore an indirect measure of amount of protein that would need to be maintained. As a result, the higher the N content, the higher the maintenance.

Several longer-term studies have been conducted during the course of a single extended dark period (Macduff and Jackson, 1992; Lee and Titus, 1993). These studies demonstrated that large differences in respiration rates occurred in the first 24 to 48 hours of a dark period, depending on temperature. However, the respiration rates tended to become equal as pools of carbohydrates were exhausted. These studies indicate that the extent of temperature dependency depends on carbohydrate concentration (Azcón-Bieto and Osmond, 1983).

Because these studies only investigate a single night period, they fail to address how a reduction in night temperature and a concomitant decrease in respiration rates influence photosynthetic rate the following day. If respiration rate decreases, insufficient protein

repair and maintenance respiration might occur during the night period, which may reduce photosynthesis the following day. An ideal system to determine the factors that control respiration would be able to measure long-term gas exchange of whole canopies, measure multiple canopies simultaneously, and be able to manipulate and control environmental conditions in each canopy separately.

Duration

Even though several studies investigated respiratory rates during long-term darkness, none of these studies continued their assessment for a subsequent day period to determine the effect of a nighttime environmental changes on photosynthetic rates and carbon use during the following day. It is hypothesized that lower night respiration rates, brought on by either an increase in temperature during the night or shade during the day, will lower the growth the subsequent day. Conversely, decreasing the night-time respiration rate will decrease the following day's photosynthetic rate. Therefore, CUE will remain close to a constant value regardless of night-time environment.

Research Objectives

The objective of this research was to understand the effect of temperature and light on respiration and the long-term control of carbon use efficiency. Specifically, I examined the effects of:

1. The role of temperature

Hypothesis 1: Respiration will double for every 10C rise in temperature.

Hypothesis 2: Warmer night temperatures will decrease photosynthesis on subsequent days because of reduced respiratory efficiency, and cooler night temperatures will increase photosynthesis on subsequent days because of improved respiratory efficiency.

Hypothesis 3: Carbon use efficiency will be similar in plants grown at constant day and night temperatures across a range of 21C to 35C.

Hypothesis 4: Carbon use efficiency will be similar in plants grown with 5C day/night difference in temperature across a range of 21C to 35C.

2. The role of substrate availability

Hypothesis 5: Elevated CO_2 will increase carbohydrate supply and decrease temperature sensitivity, whereas ambient CO_2 will decrease the supply of carbohydrates and increase temperature sensitivity.

Hypothesis 6: Shade will decrease night respiration because of decreased carbohydrate supply.

Hypothesis 7: Photosynthesis will immediately decline with low light, but respiration will require more time (days) to return to balance with photosynthesis.

3. Species

Hypothesis 8: Starch accumulators will be more sensitive to shade than sucrose accumulators because starch is a more stable storage carbohydrate causing respiration rates to remain high for longer and cause CUE to be lower.

4. Acclimation

Hypothesis 9: Carbon use efficiency will acclimate back to pretreatment values in

the days following a temperature change.

Hypothesis 10: Carbon use efficiency will acclimate back to pretreatment levels after shade is applied because respiration will decline due to lower carbohydrate supply.

Hypothesis 11: Cooler temperatures will slow carbohydrate mobilization, which will slow the rate of respiratory and CUE acclimation to low light.

Hypothesis 12: In long term studies, the maintenance respiration coefficient will be relatively insensitive to temperature due to lower respiratory demand, and growth respiration coefficient will decrease with lower light.

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Daily Carbon Gain $=$ area A - area B

Gross Photosynthesis $=$ area A + area C

Carbon Use Efficiency $= \dfrac{\text{(area A - area B)}}{\text{(area A + area C)}}$
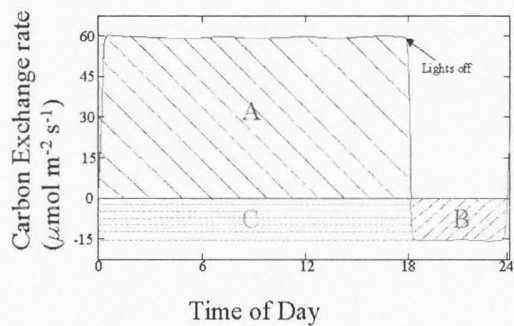
Figure 1-1. Diagram for calculating carbon use efficiency from a single day's CO_2 gas-exchange data.

CHAPTER 2

NIGHT TEMPERATURE HAS A MINIMAL EFFECT ON RESPIRATION AND

GROWTH IN RAPIDLY GROWING PLANTS[1]

Abstract

Carbon gain depends on efficient photosynthesis, adequate respiration, and adequate

respiration. The effect of temperature on photosynthetic efficiency is well understood. In

contrast, respiration is widely accepted to double with each 10C increase in temperature

(a 100% increase), but this relationship is derived from short-term (hours) measurements

in mature organisms. These short-term data are then used to extrapolate across whole life

cycles to predict the influence of temperature on plant growth. In this study, night

temperature was altered from 17 to 34C in young, rapidly growing plant communities for

up to 20 days. The day temperature was maintained at 25C. CO_2 gas-exchange was

continuously monitored in 10 separate chambers to quantify the effect of night-

temperature on respiration, photosynthesis, and the efficiency of carbon gain (carbon use

efficiency). Total respiration increased only 20 to 46% for each 10C rise in temperature.

This change resulted in only a 2 to 12% change in carbon use efficiency, and there was no

significant effect on cumulative carbon gain or dry mass. No change in the sensitivity of

respiration was observed even after 20 days of treatment. These findings indicate that

whole-plant respiration of rapidly growing plants is lower than commonly reported for

excised, mature plant tissue.

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[1]Coauthored by Bruce Bugbee.

Introduction

Plants have evolved in an environment that provides cooler nights than days. Researchers and controlled environment users often provide these conditions for plant growth, but the reasons behind this from a carbon gain perspective are not clear. Respiration may be inefficient on warm nights and inadequate on cool nights. Cool nights would reduce growth if there was not enough respiration to meet the maintenance and growth needs for the subsequent day. During warm nights, too much carbon may be inefficiently respired or predominately used on maintenance rather than on growth, thereby reducing growth rates. McCree and Amthor (1982) found that a constant 20C day/night temperature increased growth rate slightly due to improved carbon balance of a stand of white clover (*Trifolium repens*) when compared to a stand grown in 30C days and 10C nights. They suggested that the warm day temperature increased the rate of substrate use, while low respiration at night was insufficient to offset the day-time carbon loss.

Respiration is typically measured across short intervals (minutes or hours) on fully expanded leaves, leaf disks, or mature plant parts (Alexander et al., 1995; Crawford and Huxter, 1977; Bunce, 1991; Roberts et al., 1992; Labate and Leegood, 1989; Maier et al., 1998), and these measurements are used to predict plant growth. Unfortunately, it is extremely difficult to find a representative leaf. Leaves poorly represent whole plant respiration because the measurements do not include roots, stems, flowers, or meristems, and most measurements are made on mature organs that may not reflect plants with high

growth rates. Rarely, whole plants are measured, but even then, these measurements may not represent healthy plants because the respiration measurements are summed from excised roots and shoots, and are done for only a few hours (Tjoelker et al., 1999). Groups of whole plants (communities, plant stands) are also important to measure because side lighting issues from single plants makes extrapolation from single plants to whole communities complex.

There is general acceptance (based on measurements of mature tissue) of an exponential rise of respiration with temperature, and short-term studies typically indicate a respiratory Q_{10} of at least 2 (Lomander et al., 1998; Burton et al., 1996; Bustan and Goldschmidt, 1998). Unfortunately, this easy-to-use temperature correction term is used to correct total respiration measurements for differences in temperature without an understanding of what the Q_{10} sensitivity should apply to in the measurement.

Classically, respiration has been divided into a "growth" component and a "maintenance" component. The growth respiration coefficient (or efficiency of biosynthesis) has long been considered temperature insensitive (Penning de Vries et al., 1974), but maintenance respiration is considered temperature dependent (McCree, 1974). McCree (1974) described the growth portion as a function of daily photosynthesis and the maintenance portion as a function of existing biomass. Both types of respiration occur simultaneously in an actively growing plant. The theory behind this model has been supported in recent reviews (Amthor, 2000; Cannell and Thornley, 2000; Thornley and Cannell, 2000). If a large fraction of respiration is of the "growth" type, and if the efficiency of growth (or amount of growth respiration per unit growth) is temperature

insensitive, then a growing plant will be relatively less temperature sensitive and have a lower Q_{10} than a single, mature-excised leaf or root. This calls into question the practice of using measurements of respiratory sensitivity based on mature tissue to describe the carbon balance of plants that are still rapidly growing.

Amthor (2000) suggested that respiration may be less temperature sensitive during the long term. There is evidence that species acclimate to temperature and when compared at their growth temperatures, they have similar respiration rates (Q_{10} is 1), or their temperature sensitivity is less than that predicted by short-term responses (Q_{10} less than 2) (Arnone and Körner, 1997). There is, however, great species variability in how much acclimation occurs. Larigauderie and Körner (1995) found that some species acclimated to some degree, but others had long-term values of Q_{10} up to 5.5. The degree of acclimation was related to genus, which strongly suggests a genetic component to temperature acclimation.

If low Q_{10} values are calculated, does it indicate acclimation? Tjoelker et al. (2001) review many short-term studies and suggest that Q_{10} decreases as the measurement temperature increases. This tendency is easily explained mathematically when one considers that an exponential rise in respiration with temperature is more of a convenience in describing respiratory response to temperature rather than an exact behavior of respiration. Tesky and Will (1999) and Percival et al. (1996) observed a lower temperature sensitivity for whole plants than for leaves, but they attributed this to their choice of species rather than a fundamental physiological mechanism. Further confusing the matter, Griffin et al. (2002) argue that Q_{10} should actually be higher on a

whole plant basis than lower due to a reduction in the likelihood of becoming carbohydrate limiting during measurement at warm temperatures.

In addition to measuring respiration rates, it is useful to get a measure of whole-plant respiratory efficiency and carbon conservation. A widely used parameter for this is the ratio of net carbon gain (net photosynthesis - dark respiration) in a 24-h period to the total carbon fixed during the light period (P_{gross}) (Amthor, 1989). This ratio, called carbon use efficiency (CUE), is a measure of the efficiency of incorporation of fixed carbon into new biomass. The term integrates all growth and respiratory processes in a plant during a 24-h period. CUE should be affected by temperature and growth rates. Understanding environmental effects on respiration is critical to understanding CUE.

Surprisingly, some studies have found that CUE changes little during a plant's life cycle in spite of large differences in temperature and growth rates. Gifford (1994) reported an average CUE (calculated from his R:P ratio) of 0.58 to 0.60 for several species and sizes of plants across a wide range of environments, which calls into question the temperature sensitivity of CUE. Monje and Bugbee (1998) found a CUE for wheat (*Triticum aestivum* L.)of 0.59 to 0.61 except for the first and final week of the life cycle. Dewar et al. (1998) modeled CUE of mature canopies and suggested it should be constant because stored reserves should buffer short-term changes in substrate availability.

We examined both the short-term (days) and long-term (weeks) effects of temperature on respiration, net photosynthesis, and CUE of groups of whole plants. We minimized the effect of temperature on photosynthesis by maintaining a constant daytime temperature, and we minimized temperature effects on photorespiration by growing the

plants at elevated CO_2. We also minimized the effect of temperature on leaf expansion by studying groups of plants and imposing temperature treatments when canopies were uniform. We hypothesized that changing night temperature would exert a strong influence on night respiration and that change would result in differences in photosynthetic rates of the subsequent day. Furthermore, we hypothesized that altered night respiration would result in a change in CUE, but that both respiration and CUE would acclimate to their pre-treatment levels.

Materials and Methods

Experimental Setup and Design

Three studies were conducted to compare night-temperature effects across three crops: lettuce (*Latuca sativa* L. 'Grand Rapids'), tomato (*Lycopersicum esculentum* Mill. 'Red Robin') and soybean (*Glycine max* (L.) Merrill 'Hoyt'). Seedlings were transplanted four to seven days after imbibition into a 10-chamber, computer-controlled gas-exchange system (Figure 2-1). Temperature within each chamber was controlled with a chilled water coil and small heaters. System details and calibration procedures were described previously (van Iersel and Bugbee, 2000). Lettuce and tomato were grown at constant 25C, and soybeans were grown at a constant 20C until canopy closure. Daytime temperatures remained at these temperatures for the duration of the trial. Temperatures were measured with an aspirated, type-E (0.5 mm diameter, 24-AWG) thermocouple, and were maintained within ±0.2C of the set point. Groups of whole plants or plant communities were studied and arranged at the following densities: lettuce at 106 plants

m^{-2}, tomato at 80 plants m^{-2}, and soybean at 35 plants m^{-2}.

Night temperatures were controlled across a 17C range from 17 to 34C. Temperature treatments were maintained for 13 days until harvest on Day 29 for lettuce, for 20 days until Day 36 for tomato, and for 15 days until Day 34 for soybeans. Treatments extended into flowering for tomato and soybeans. Control plants had constant day/night temperature. All days are after transplanting.

Relative humidity was maintained between 60 and 80%, but varied much less among the chambers for any given day because the daytime environments were similar (data not shown). A photosynthetic photon flux (PPF) of 600 μmol m^{-2} s^{-1} ($\pm$5% among chambers) was provided by water-filtered, high pressure sodium lamps. The photoperiod was 16-h for lettuce and tomato, and 12-h for soybeans. Reflective material was wrapped around each chamber and was adjusted daily to the top of the canopy to minimize side lighting (Figure 2-1). CO_2 was controlled at 1,200 μmol mol^{-1} for the duration of all the studies. These studies were conducted at elevated CO_2 for three reasons: 1) to minimize photorespiration, 2) to minimize the effect that vapor pressure deficit differences would have on photosynthesis if there were differences between chambers on a given day, and 3) to increase photosynthesis and thus help insure that the plants did not become carbohydrate limited at the higher temperatures. Temperature responses likely would be smaller if the plants were carbohydrate limited by ambient CO_2. Separate hydroponic systems were enclosed in each chamber so that root respiration was included with the shoot. Hydroponic solution was bubbled with the same air as that used for the shoot environment.

Manual adjustment of pH on a daily basis resulted in a one pH unit day-to-day range. The pKa of carbonate is 6.2, which means that 50% of the carbon dissolved in the water is in the carbonate form and 50% is CO_2. Due to the limitations of our pH control method, a one pH unit range during the day has the potential to cause significant fluxes in and out of the nutrient solution. For this reason, the pH of the hydroponic solution was maintained between 4 and 5, which forces between 90 and 99% of the CO_2 out of solution.

Calculations

Carbon use efficiency (CUE) is a calculated term that measures how efficiently plants can incorporate the carbon fixed during the day into biomass gain. It is used instead of percent respiration because: 1) it is an efficiency parameter that can easily fit within models, and 2) it incorporates differences in photoperiod automatically, whereas percent respiration does not. Using P_{net} (net photosynthesis, mol C m_{ground}^{-2} d^{-1}) and R_n (night-time respiration, mol C m_{ground}^{-2} $night^{-1}$), daily carbon gain (DCG) can be calculated as:

$$DCG = P_{net} - R_n$$

Cumulative carbon gain (CCG) is the running total of DCG.

P_{gross} is a calculated term that reflects the net C fixed (P_{net}) and the amount of C that is simultaneously being respired. Because day-time respiration (R_d) can not be measured directly, P_{gross} is expressed as the sum of P_{net} and some percentage of R_n. Some studies have indicated that R_d in leaves can be higher during the day due to their higher daytime carbohydrate content (Azcon-Bieto et al., 1983; Azcon-Bieto and Osmond, 1983). Other

studies indicate daytime R_d is lower due to light-inhibition of respiration (Sharp et al., 1984; Wang et al., 2001). Monje and Bugbee (1996) found that root respiration, at a constant temperature, is increased in the day presumably due to increased carbohydrate supply. The common approach for whole plants is to assume that the rate of R_d and R_n (μmol m_{ground}^{-2} s^{-1}) are equal when temperatures are constant. In a 12-h photoperiod, R_d (mol C m_{ground}^{-2} d^{-1}) then equals Rn. In a 16-h light / 8-h dark photoperiod, $R_d = R_n * 2$. In these equations, respiration assumes a positive value (i.e., mass respired). P_{gross} (mol C m_{ground}^{-2} d^{-1}) can, therefore, be calculated as:

$$P_{gross} = P_{net} + R_d$$

Plants were grown in constant day/night temperatures until treatments were applied. Treatment effects were expressed as a percent of their initial value, then normalized to the control in the following manner:

$$(posttreatment_a \ day_b \ / \ pretreatment_a \ value) \div$$

$$(posttreatment_{control} \ day_b \ / \ pretreatment \ value_{control}) \times 100(\%)$$

where posttreatment$_a$ indicates the posttreatment value of parameter a (i.e., CUE, P_{net}, Rdark), day$_b$ indicates the day after treatment, pretreatment$_a$ value is the value of the parameter of interest on the day before treatments began, posttreatment$_{control}$ day$_b$ is the posttreatment value of the parameter of interest on the same posttreatment day, and pretreatment value$_{control}$ is the pretreatment value of the parameter of interest the day before treatments began. Temperature effects through time are measured in the numerator, and the effects relative to the control and through time are accounted for by normalizing to the denominator. Finally, the Y-intercept was adjusted to facilitate

comparisons of slopes among treatments.

To compare our temperature sensitivities to the literature and to calculate the R_d from R_n to determine P_{gross}, Q_{10} values are reported. The R_n of plants in different temperatures were used to calculate two Q_{10} values (one from control temperature to coolest temperature and one from the control temperature to the warmest temperature) using the temperature response function:

$$Q_{10} = e^{\frac{(R_n T - R_n control)}{(T - control\ T\ /\ 10)}}$$

In this study, the temperature response was not obviously exponential, and so was explained statistically with linear regression.

Statistical Analysis

A randomized block design was used with five treatments in each of two blocks, giving two replicates at each temperature. Occasionally, temperature control at the low or high treatments was not perfect. This caused us to use linear regression analysis and treat temperature as a continuous variable rather than as discrete treatments. Slopes of lines were compared to see if their slopes were equal using the test statistic ((slope a - slope b) - 0)/(variance of slope a) = t $_{(degrees\ of\ freedom\ of\ slope\ a)}$ (Neter et al., 1996).

Results

Lettuce

Night respiration increased 2.0% per degree C (F = 440.0, df = 9, P < 0.0001;

Figure 2-2A, 2-3A). After 13 days of treatment, the slopes did not differ significantly

from one another indicating no acclimation to temperature. The average CUE was 0.62

the day before treatments were imposed (Figure 2-2B). Net photosynthesis was not

sensitive to night temperature (F = 0.41, df = 9, P = 0.54; Figure 2-3B). The CUE values

for the coolest night temperatures were about 2% higher than the control values and the

warmest were about 2% lower than the control values for all days after treatments were

applied (F = 31.5, df = 9, P = 0.0005; Figure 2-3C). The sensitivity of CUE to

temperature did not change during the 13 days of treatment based on comparison of

slopes (P 0.35, df = 8). There was no difference in final dry mass (F = 1.19, df = 9, P =

0.31) or in CCG (F = 0.23, df = 9, P = 0.64) after treatments were imposed (Table 2-1).

Tomato

Night respiration increased 2.7% per degree C with warmer nights (F = 185.2, df =

9, P < 0.0001; Figure 2-4A and 2-5A). This caused the CUE to be slightly higher with

cooler nights and lower with warmer nights (F = 35.8, df = 9, P < 0.0003; Figure 2-4B

and 2-5C). After 20 days of temperature treatment, there was no difference in the

sensitivity of CUE or respiration to altered night temperature (P = 0.48, df = 8). Net

photosynthesis was not affected by the altered night temperatures (F = 0.47, df = 9, P =

0.57; Figure 2-5B). There were no differences in the final dry mass (F = 0.43, df = 9, P =

0.53) of the treatments or in CCG (F = 0.28, df = 9, P = 0.61) after treatments began

(Table 2-1).

Soybean

Soybean was the most sensitive of the three crops studied. Respiration increased 4.0% per degree C (F = 164.3, df = 9, P < 0.0001; Figure 2-6A and 2-7B). Carbon use efficiency changed after the night temperatures were changed because respiration changed (Figure 2-6B, 2-7A, and 2-7C). With no change in photosynthesis, the decrease in respiration resulted in an increase of CUE of 3% relative to the control and a decrease of 12% relative to the control at the highest temperature (F = 27.3, df = 9, P < 0.0008; Figure 2-7C). Net photosynthesis was not affected by the altered night temperatures (F = 0.056, df = 9, P < 0.82; Figure 2-7B). No acclimation occurred after 15 days of treatment (P = 0.29, df = 8). No differences in final dry mass (F = 0.0035, df = 9, P = 0.95) were observed between treatments or in the CCG (F = 1.09, df = 9, P = 0.33) after treatments began (Table 2-1).

Differential Temperature Effects
Among Species

The effect of temperature on respiration and CUE differed among species (Figure 2-8A and 2-8B). Soybean respiration was significantly more sensitive than lettuce (t = 6.34, df = 8, P < 0.001), and had more CUE sensitivity than lettuce (t = 2.91, df = 8, P < 0.025), but all three species were much less sensitive than commonly believed. Lettuce respiration was significantly less sensitive than tomato (t = 3.418, df = 8, P < 0.025), and CUE was marginally significantly different (t = 1.64, df = 8, P ~ 0.08). The average Q_{10} for whole plant respiration of lettuce was 1.20, tomato was 1.36, and soybean was 1.46, with lower Q_{10} values for warmer temperatures (Table 2-1).

Discussion

Current Paradigm: Respiration has a Q_{10}
of about 2

The third edition of a popular plant physiology textbook states that respiration

typically doubles for every 10C rise in temperature between 10 and 30C (Taiz and Zeiger,

2002), and this is a common approximation for respiration response to temperature based

on short-term (minutes or hours) measurements made on mature plant parts. However,

values of Q_{10} for respiration in plants, animals, and microbes are reported from 1.2 to 4

(Urmeneta et al., 1998; Neven, 1998; Boone et al., 1998; Quinlan and Lighton, 1999;

Lariguderie and Körner, 1995; Chapman and Thurlow, 1998; Nielsen et al., 1999). Some

have speculated that the range in Q_{10} values is the result of acclimation to temperature, or

simply a result of measurement temperature. Because the growth coefficient for

respiration is believed to not differ with temperature, it stands to reason that if a large

fraction of the respiration is growth respiration, the temperature sensitivity for total

respiration would be less than values obtained from mature (not growing) tissue. In our

studies, small, rapidly growing plants were used, which suggests that a large fraction of

the respiration is likely growth respiration, and could explain the low Q_{10} values obtained

in this study.

Studies that report low values for Q_{10} have all had some degree of growth of the

organism, which would result in a temperature-insensitive growth component included in

the respiration measurements, though they do not separate growth and maintenance

components (Ogawa and Takano, 1997; Percival et al., 1996; Urmenta et al., 1998). The

studies that have less growth have values of 2 to 2.5 (Maier et al., 1998; Quinlan and Lighton, 1999; Neven, 1998), regardless of the organism. Models and modeling literature do an excellent job of separating growth and maintenance respiration and assigning the temperature sensitive portion only to the maintenance fraction. Unfortunately, studies investigating the effect of temperature on respiration too often fail to divide respiration into its component parts and, as a result, conclude that a given species may have an unusually low temperature sensitivity.

If we assume that the Q_{10} for maintenance respiration is between 2 and 2.2, maintenance and growth coefficients can be calculated. Assuming all of the measured temperature sensitivity was due to maintenance respiration, the maintenance coefficient is 0.03 to 0.04 mol C respired per mol of C in biomass per day, which is a typical value (Amthor, 1989). It follows that the remainder of the respiration is due to growth, which results in a conversion efficiency (sometimes referred to as Yg) of 0.85 to 0.95 mol C in growth per mol substrate, which is a realistic value based on estimates of Penning de Vries et al. (1974). These calculations also indicate that 34 to 50% of the respiration is growth respiration, which is a reasonable value for these young plants (van Iersel and Seymour, 2000).

Current Paradigm: Cool night
temperatures improve growth

McCree and Amthor (1982) found that growth rate was improved by 15% at a constant 20C compared to 30/10C day/night due to improved carbon balance. They attributed the growth improvement to excessive dark respiration during the day and only

slightly reduced night-time respiration, but they used ambient CO_2 and photorespiration at

30C would thus be significantly increased compared to 20C. This would decrease

photosynthetic efficiency and reduce growth in the warmer day temperature treatment.

They also used a 20C difference between day and night. This extreme change in

temperature may have affected water relations, leaf expansion, and chilling injury in

addition to respiration so the direct effect of night temperature on growth is not clear from

their study.

In theory, respiration may be inadequate on cool nights and excessive on warm

nights, but our studies do not indicate a statistically significant advantage of either warm

or cool nights on final dry mass or on cumulative carbon gain after treatments were

applied. There was a statistically significant effect of temperature on night respiration, as

expected, but, surprisingly, there was no significant effect on P_{net}. Because the carbon

flux in P_{net} is typically 4 to 5 times larger than dark respiration in growing plants, changes

in dark respiration have only a small effect on CUE. The relatively small variation

between replicate chambers in P_{net} dominated the effect of the small, but statistically

significant, differences in CUE on growth. If experimental error in P_{net} was eliminated,

the growth effects would have been determined by the night temperature effect on CUE,

which was less than 0.5% per degree C in lettuce and tomatoes, and less than 1% per

degree C in soybeans. These effects do suggest a small advantage of cool night

temperatures that might be statistically significant in a study with many replicate

chambers, but our data indicate that cool nights are biologically insignificant.

Current Paradigm: Respiration and CUE
acclimate to temperature changes

Although both respiration and CUE were influenced by temperature, neither

returned to pre-treatment levels after treatments began. Several studies reported

respiratory acclimation or adaptation to changes in temperature, and some back to

pre-treatment levels (Spencer and Wetzel, 1993; Illeperuma et al., 1998; Larigauderie and

Körner, 1995). Gifford (1995) found that CUE returned to the pre-treatment level within

four days. The initial change in CUE in our study was similar to Gifford (1994), but he

altered both day and night temperature and grew his plants in ambient CO_2, so changes in

photorespiration may have complicated the results.

Tjoelker et al. (1999) argue that a lower Q_{10} than that obtained from short-term

measurements indicates acclimation to temperature. Detached plants were used in that

study, so one could argue that a lower Q_{10} was more a result of measuring dead plant parts

rather than acclimation. Still, a lower Q_{10} suggests a decrease in temperature sensitivity,

so did the plants in our study acclimate? Ideally, both short and long-term measurements

of temperature sensitivity on a plant could be compared. However, making those

measurements on the same plants would compromise the integrity of any 'constant night

temperature' treatment, as used in this study. If the only requirement for acclimation is a

lower Q_{10} (than expected), then these plants acclimated. However, the temperature

sensitivity did not change during the entire treatment period (up to 20 days), so any

acclimation must have occurred during the first day only and not acclimated any more.

This seems unlikely.

Another possibility is that the low Q_{10} was due to substrate limitation; if there is no substrate to respire, then respiration cannot increase with temperature. These plants were grown in 1,200 μmol CO_2 mol^{-1} with relatively high light (34.5 mol photons m^{-2} d^{-1}). Previous studies done in similar environments indicated ample carbohydrate supply (Smart et al., 1994). If anything, the plants in this study should have been more sensitive to temperature than an average greenhouse grown or field grown plant. The fact that they had such small sensitivities suggests that other mechanisms are at work (i.e., growth respiration is a large fraction of total respiration).

It is unlikely that decreases in daytime respiration compensated for increases in night respiration. For example, the night respiration of soybean increased from 1.0 to 1.4 mol C m^{-2} $night^{-1}$ when the night temperature was increased from 20 to 30C. The day-time respiration would need to decrease by 75% for CUE to remain the same. There was no effect of temperature on P_{net} so this should result in a 12% decrease in P_{gross}. Also, this type of change would indicate an uncoupling of temperature and respiration during the day as well as a decrease in respiration with an increase in carbohydrate supply, which contradicts the literature (Azcón-Bieto and Osmond, 1983; Azcón-Bieto et al., 1983; Moser et al. 1982; Monje and Bugbee, 1996). While a 75% reduction in day-time respiration may be possible in single leaves, there is no evidence on a whole plant level that this occurs. Furthermore, this type of acclimation seems unlikely, and there is no known reason why this would occur.

Dewar et al. (1998) developed a mechanistic model for mature leaves or canopies of CUE to explain why constant short-term (days) CUE is often observed in many species

and environmental conditions. In this model, stored reserves and available carbohydrate for growth and respiration are balanced across several days. The balance of these pools is nearly steady when averaged across several days so brief fluctuations (minutes or hours) in environments are not believed to affect the balance. The model was tested in variable light conditions, but variation in temperature was not modeled. The pool of carbohydrate available for growth and respiration is typically large enough to negate small, temporary changes in the environment and the result is constant CUE. Furthermore, the synthesis of starch and proteins is maintained in an approximately steady state during these temporary fluctuations, so while the plant may grow at different rates in response to more or less light, it should have identical growth efficiency. That does not mean, however, that CUE should also remain constant.

The crops in our study developed in one environment, which was suddenly changed for the duration of the study. The pools of carbohydrate available for growth and respiration may have been permanently altered, thereby resulting in a new CUE value. It is possible that the new biomass (carbohydrate, lipids, proteins) was also affected by this change and, as a result, the CUE changed.

CUE has been modeled to be a function of conversion efficiency (Y_g), the maintenance coefficient, and relative growth rate (RGR) (Thornley and Johnson, 2000). According to this model, CUE should decrease as RGR decreases. However, because the model uses a hyperbolic-type equation similar to the Michaelis-Menton enzyme kinetics equation, RGR can vary greatly without substantial change in CUE. Interestingly, Thornley and Cannell (2000) ignore the RGR:CUE relationship and state that one of the

results from mechanistic analyses of respiration and growth should be a constant CUE.

Applicability to Ambient CO_2
Environments

There is concern that physiological studies, and especially respiration studies, can not be applied to most situations if the studies were done in elevated CO_2. One of the primary reasons this study was performed in elevated CO_2 was to minimize the probability of respiration becoming substrate limited in warm temperatures. Reduced carbohydrate availability in lower CO_2 would likely make the temperature response even smaller than what we measured.

Some previous studies suggested that elevated CO_2 inhibited respiration. For example, Amthor et al. (1992) initially reported a 20 to 30% reduction in dark respiration when CO_2 was doubled. However, no theoretical basis for the effect of CO_2 on dark respiration has been found. Recent studies indicated that the direct effect of CO_2 on dark respiration is statistically insignificant and appears to be biologically unimportant. Amthor (2000) used an improved gas exchange chamber and found that the apparent direct effect of CO_2 on respiration resulted from leaks in the original chamber. Indeed, using five gas exchange measurement approaches, he consistently found that respiration was insensitive to short-term changes in CO_2 concentration. Similarly, Burton et al. (1996) initially reported a significant inhibition of root respiration in elevated CO_2, but later re-did the tests and found that once leaks were sealed, no CO_2 effect was observed (Burton and Pregitzer, 2002). It is even questionable whether there is a long-term effect of elevated CO_2 on specific respiration rates. Monje and Bugbee (1998) compared specific

respiration rates at high and low CO_2 (1,200 vs. 400 µmol mol^{-1}) and found a small difference during the first 6 days of growth, but no difference during the remainder of the life cycle.

In summary, respiration had a much lower Q_{10} than is commonly used in studies investigating the effect of temperature on respiration. Whole plant respiration is relatively insensitive to temperature in young plants because total respiration consists of a large fraction of total respiration. In light of this, the present work validates models that divide respiration into a non-temperature sensitive growth component and a temperature-sensitive component. No change in the sensitivity of respiration to temperature was observed even after 20 days of treatment. Because of the lack of acclimation of respiration to temperature through time, CUE also did not adapt. We believe this is the first long-term study to demonstrate continuously altered CUE as a result of environmental changes.

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Table 2-1. Final dry mass (in g per m^2 ground area), cummulative carbon gain (CCG) after treatment initiation, and respiration sensitivity to temperature, as measured by Q_{10} for each species. Data are the average of two groups of plants in separate chambers. Q_{10} was higher at cooler temperatures and lower in warmer temperatures. There was no effect of temperature on final dry mass in any species (P>0.32), nor on CCG (P>0.59).

Crop	Night temp.	Final plant mass	CCG after treatment initiation	Sensitivity to temperature
	(C)	$(g\ m^{-2})$	$(mol\ C\ m^{-2})$	(Q_{10})
Lettuce	18.2	476.8	13.8	1.22 to 1.19
	20	466.7	14.3	
	25	483.7	13.5	
	30	493.0	13.9	
	31.3	490.5	13.8	
Tomato	18	768.3	22.8	1.38 to 1.34
	20	730.8	20.8	
	25	740.0	21.8	
	30	784.1	23.0	
	31.5	751.6	22.4	
Soybean	17	901.0	22.8	1.61 to 1.40
	20	881.3	23.2	
	25	927.5	23.0	
	30	842.8	23.6	
	32	879.8	23.1	
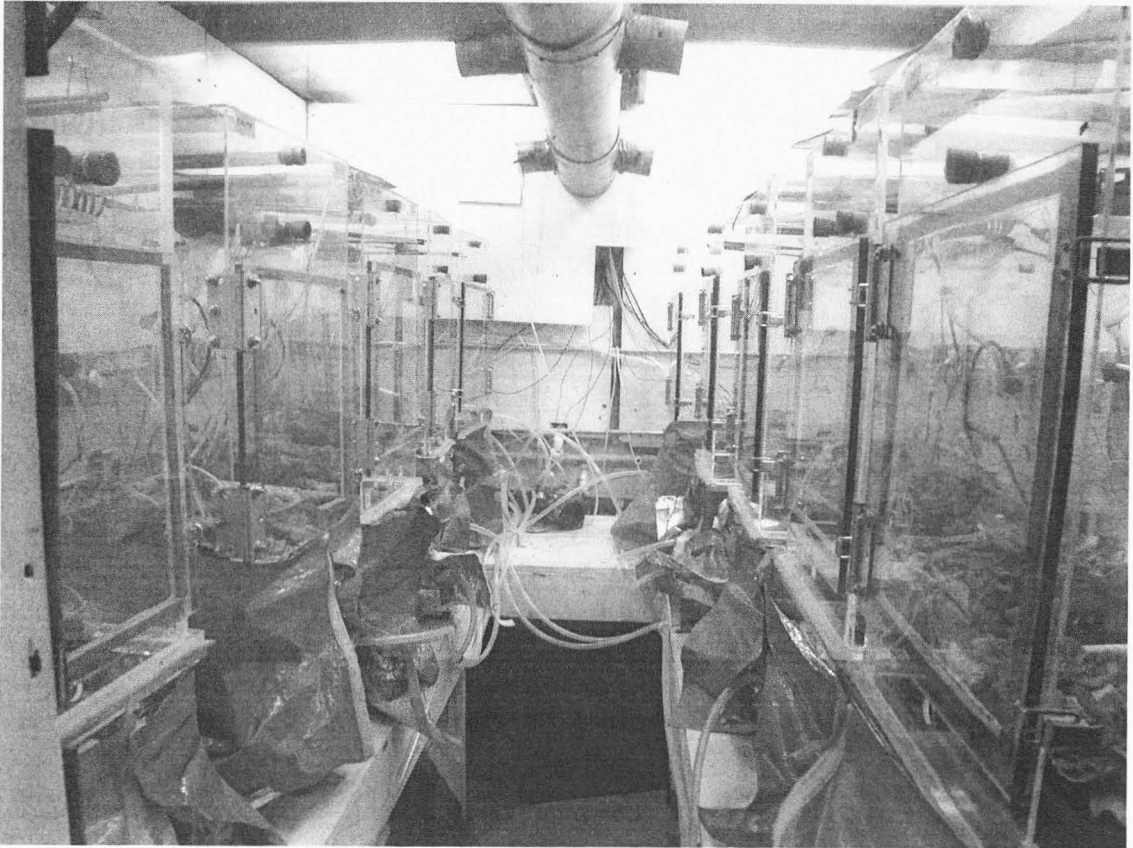
Figure 2-1. The ten-chamber gas-exchange system. There are five chambers on each side of a walk-in growth chamber. Each chamber has a reflective skirt wrapped around the outside to minimize side lighting.
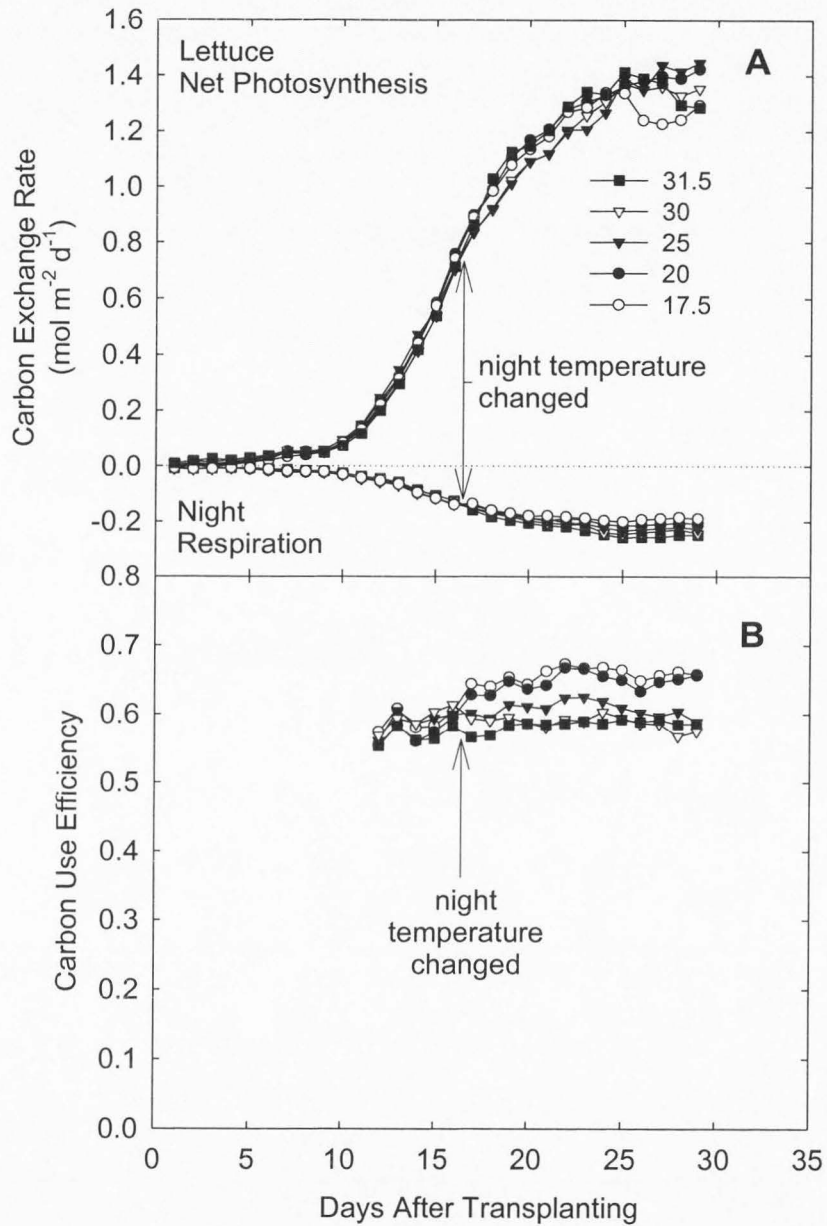
Figure 2-2. Net photosynthesis, night respiration (A), and carbon use efficiency (B) for

lettuce. Data points are the average of two chambers for a given temperature. Carbon

exchange rates are per unit ground area. Temperature treatments were initiated on Day

17.
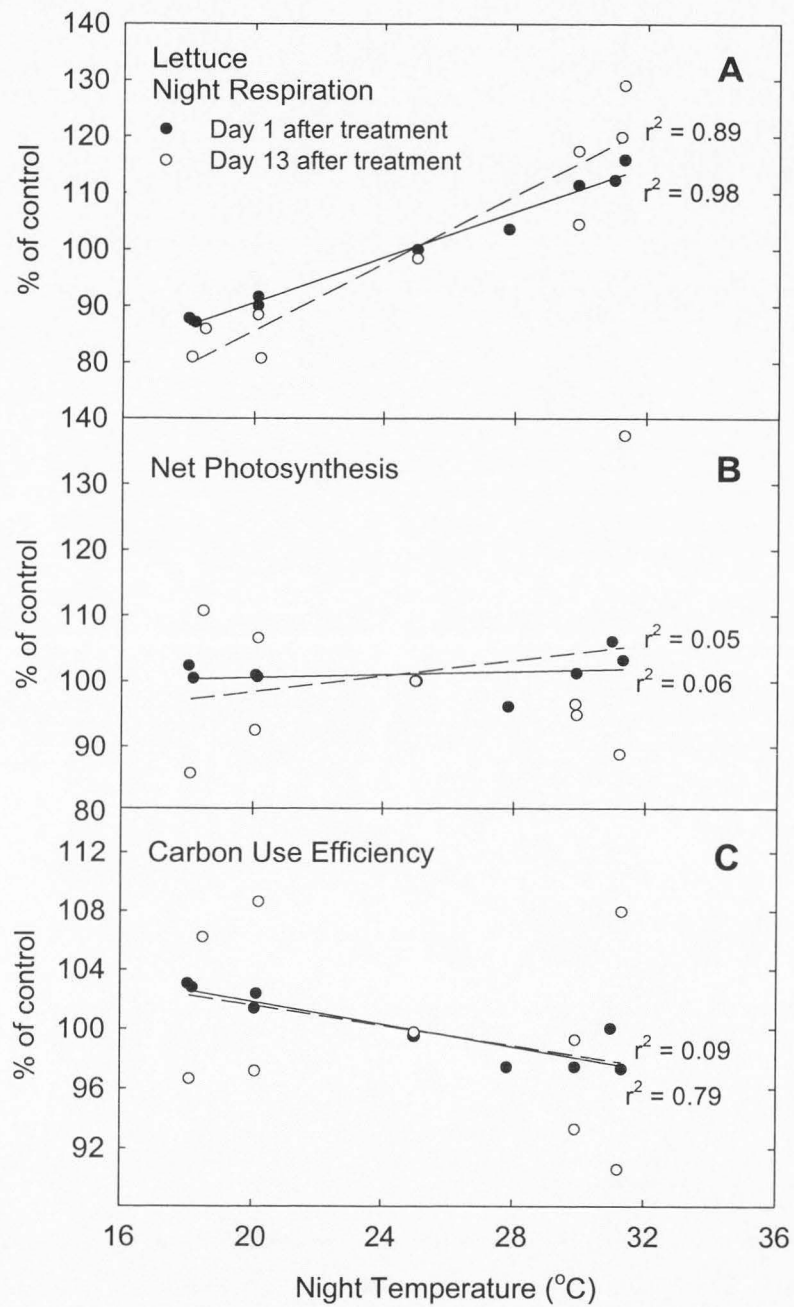
Figure 2-3. Effect of temperature on night respiration (A), photosynthesis (B), and carbon use efficiency (C) for lettuce. Data are shown relative to the control for the first and last day of treatment. The slopes are not statistically different from one another (lowest P value 0.35, df = 8) indicating no acclimation to temperature.
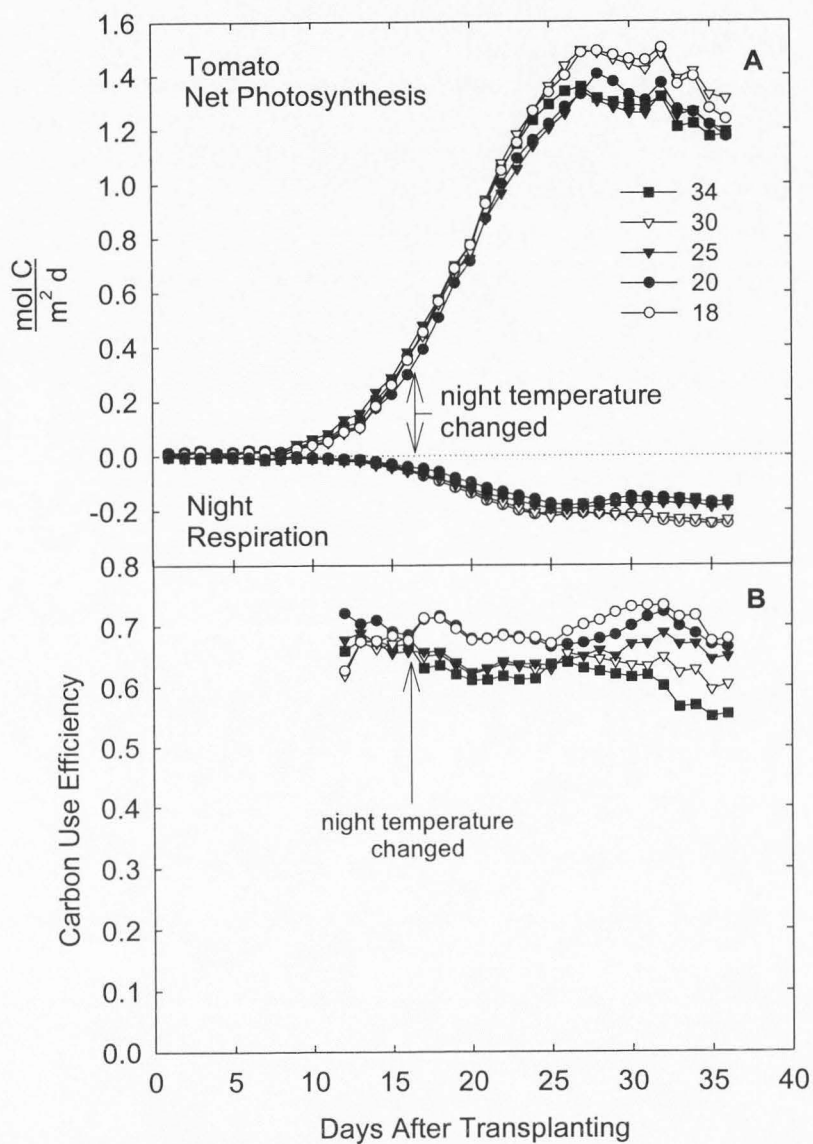
Figure 2-4. Net photosynthesis, night respiration (A), and carbon use efficiency (B) for tomato. Data points are the average of two chambers for a given temperature. Carbon exchange rates are per unit ground area. Temperature treatments were initiated on Day 17.
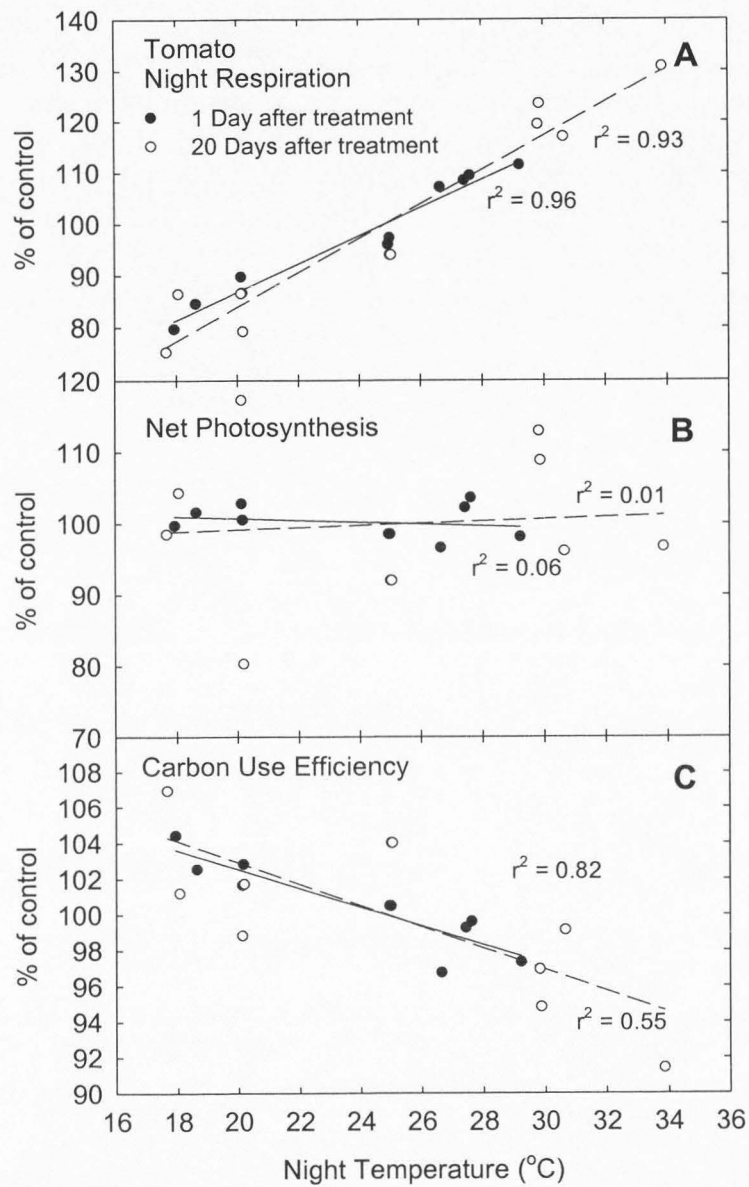
Figure 2-5. Effect of temperature on night respiration (A), photosynthesis (B), and carbon use efficiency (C) in tomato. Data are shown relative to the control for the first and last day of treatment. The slopes are not statistically different from one another (lowest P value 0.48, df = 8) indicating no acclimation to temperature.
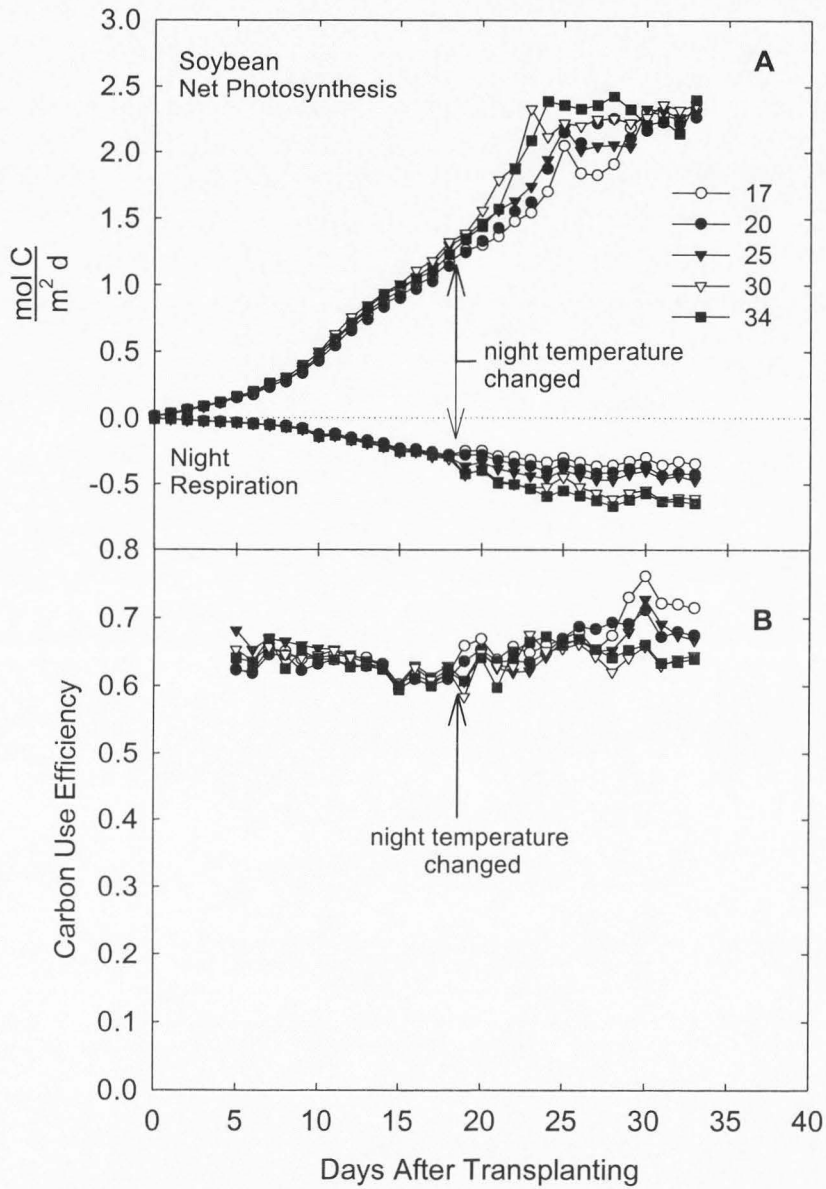
Figure 2-6. Net photosynthesis, night-time respiration (A), and carbon use efficiency (B)

for soybean. Data points are the average of two chambers for a given temperature.

Carbon exchange rates are per unit ground area. Temperature treatments were initiated

on Day 18.
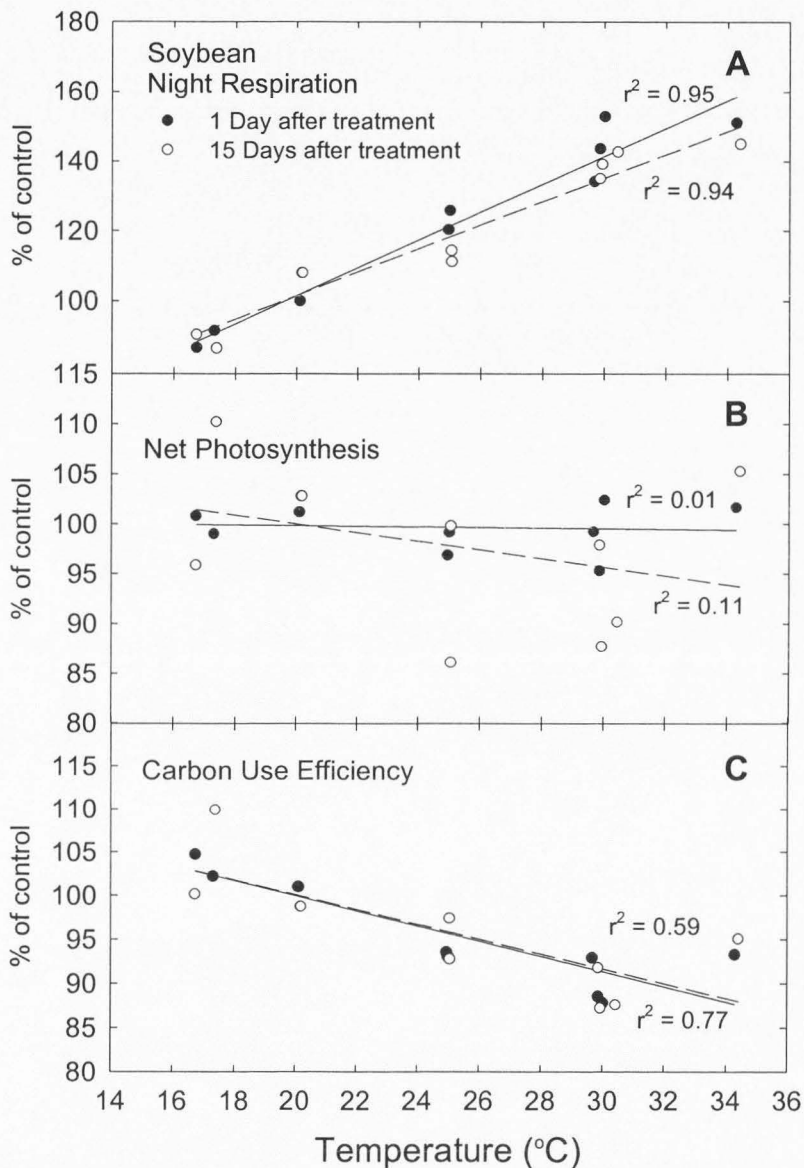
Figure 2-7. Effect of temperature on night respiration (A), photosynthesis (B), and carbon use efficiency (C) in soybean. Data are shown relative to the control for the first and last day of treatment. The slopes are not statistically different from one another (lowest P value 0.29, df = 8) indicating no acclimation to temperature.
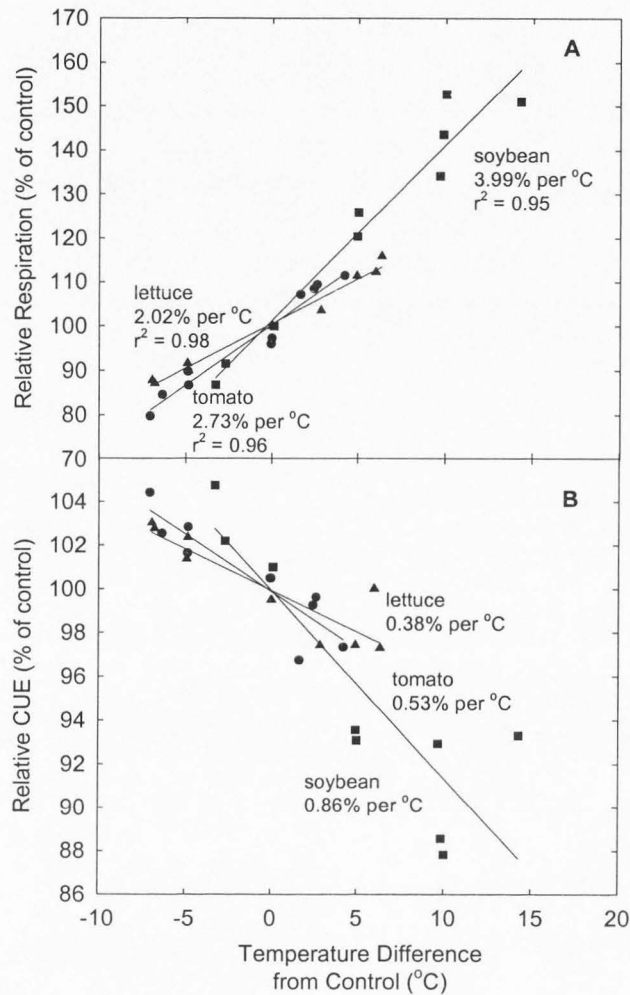
Figure 2-8. Effect of temperature on respiration (A) and carbon use efficiency (B) in soybean, lettuce, and tomato relative to the control. Soybean respiration was significantly more temperature sensitive than tomato (t = 4.05, df = 8, P < 0.005), and tomato respiration is significantly more sensitive than lettuce (t = 3.418, df = 8, P < 0.025). Soybean CUE was significantly more sensitive to temperature than tomato (t = 2.04, df = 8, P < 0.05), and tomato CUE was marginally more sensitive to temperature than lettuce (t = 1.64, df = 8, P ~ 0.08). Data are normalized to the control temperature (25C in lettuce and tomato, and 20C in soybean).

CHAPTER 3

RADIATION CAPTURE, PHOTOSYNTHESIS, AND

RESPIRATION AS AFFECTED BY TEMPERATURE AND CO_2[2]

Abstract

The effect of day/night temperature on photosynthesis, respiration and the balance between the two has not been well studied. It has been hypothesized that growth responses to temperature may be the result of differences in carbohydrate supply and demand. Therefore, CO_2 concentration may influence the effect of temperature. Using whole canopy CO_2 gas exchange, four trials were run to test the effect of constant temperature, or a 5C day/night temperature difference at two CO_2 concentrations (ambient or 400 µmol mol^{-1} and elevated or 1,200 µmol mol^{-1}) in lettuce (*Latuca sativa*) communities. Temperature greatly influenced the rate of leaf emergence and expansion, which resulted in different levels of radiation capture. Concentration of CO_2 had a minor effect on radiation capture, with canopies grown in higher CO_2 concentrations reaching canopy closure about three days earlier than ambient CO_2-grown canopies. Canopy photosynthetic efficiency (quantum yield) decreased with increasing temperature in ambient CO_2 canopies, but increased with temperature in elevated CO_2. Carbon use efficiency was not affected by temperature in elevated CO_2, but decreased at the highest temperature in low CO_2. Canopies tended to respond to the average daily temperature rather than high or low temperature during a 24-h period for carbon gain, radiation capture, quantum yield, and carbon use efficiency.

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[2]Coauthored by Bruce Bugbee.

Introduction

Plants evolved in environments that have warm daytime and cool night-time temperatures. Often, users of controlled environment chambers or greenhouses control temperature to simulate natural temperature fluctuations. Commercially, growers have reversed this to provide warm night temperatures and cool day temperatures to reduce plant height in the greenhouse floriculture industry. This practice is called DIF (Erwin and Heins, 1990; Kaufmann et al., 2000). Given the common experimental and environmental conditions as well as the commercial importance of maintaining warmer nights than days, the effect of day/night temperature on growth has not been well studied. It has been hypothesized that cool night temperatures conserve carbon for the plant through a reduction in respiration in response to temperature. McCree and Amthor (1982) published one of the only papers on the metabolic effects of altered day/night temperature. They found that growth of a stand of white clover (*Trifolium repens*) at a constant 20C day/night temperature was slightly improved compared to a canopy grown at 30C days and 10C nights. They suggested that warm day temperatures increased the rate of substrate use, while low respiration at night was insufficient to offset the daytime carbon loss.

Other factors may influence plant growth besides respiration. Sometimes called the determinants of growth, radiation capture efficiency, photosynthetic efficiency (quantum yield), and respiration efficiency (carbon use efficiency) all potentially play a significant role in the determination of plant growth. In spite of their importance, these factors are

rarely measured together and on a whole plant or canopy basis.

Leaf expansion rate and, therefore, radiation capture, is greatly influenced by temperature (Faust and Heins, 1994; Clifton-Brown and Jones, 1997) and along with leaf emergence rate, provide a plant the means to effectively capture light as it develops from a seedling. The temperature optimum for leaf expansion differs for different crops. Cotton (*Gossypium* spp.), a warm-season crop, has a temperature optimum for leaf expansion of about 31C (Reddy et al., 1993), while broccoli (*Brassica oleracea* L. italica) has an optimum of only 21C (Olesen and Grevsen, 1997). Lettuce (*Latuca sativa*) is considered to be a cool-season crop, and while the temperature optimum for leaf expansion and radiation capture is not known, the optimum for growth is reportedly 19 to 23C day and 7 to 11C night (Swaider et al., 1992). The optimum for leaf expansion is hypothesized to be in the same range.

Quantum yield (QY) is a measure of how efficient the photosynthetic machinery is at fixing CO_2 with available light. Photorespiration, a competing reaction that fixes O_2 instead of CO_2, increases with temperature for C3 crops, and reduces QY in warmer temperatures in ambient CO_2. Photorespiration can be significantly reduced at $[CO_2]$ of 1,200 $\mu mol\ mol^{-1}$ so that electron transport becomes the rate limiting factor in CO_2 fixation. The potential rate of electron transport continues to increase up to 30 to 35C (Farquhar et al., 1980). Therefore, the temperature optimum for QY of C3 species should increase several degrees under elevated CO_2 (Harley and Tenhunen, 1991; Long, 1991).

A useful and widely used indicator of whole-plant respiratory efficiency is the ratio of net carbon gain (net photosynthesis - dark respiration) in a 24-h period to the total

carbon fixed during the light period (P_{gross}) (Amthor, 1989). This ratio, called carbon use efficiency (CUE), is a measure of the efficiency of incorporation of fixed carbon into new biomass. The term integrates all growth and respiratory processes in a plant during a 24-h period.

Surprisingly, some studies have found that CUE changes little during a plant's life cycle despite large differences in temperature and growth rates. Gifford (1994) reported an average CUE of 0.58 to 0.60 for several species and sizes of plants. Monje and Bugbee (1998) found a CUE for wheat of 0.59 to 0.61 except for the first and final week of the life cycle. Dewar et al. (1998) modeled CUE and suggested it should be constant because stored reserves should buffer short-term changes in substrate availability. In Chapter 2 we altered only night temperatures in elevated CO_2 and found that CUE was minimally affected due to a much smaller respiration response to temperature than previously reported.

In this study, I examined the long-term (weeks) effects of temperature on radiation capture efficiency, photosynthetic efficiency, and CUE. I also examined how CO_2 influences each of these factors. I hypothesized that temperature would influence radiation capture with the optimum temperature being about 25C. Elevated CO_2 would not influence the rate of leaf expansion, but would improve the photosynthetic efficiency, thereby, shifting the optimum temperature a few degrees warmer. Finally, I hypothesized that CUE would remain constant regardless of the treatment.

Materials and Methods

Experimental Setup and Design

Lettuce (*Latuca sativa* L. 'Grand Rapids') seedlings were germinated on blotter paper and transplanted four days after imbibition into a 10-chamber gas-exchange system (van Iersel and Bugbee, 2000). Each chamber is 0.5 x 0.4 x 0.9 (L x W x H). Seedlings were arranged uniformly within each chamber at a density of 106 plants m^{-2}. Separate hydroponic systems fit entirely inside each chamber. Hydroponic solution was bubbled with the same air as that used in the shoot environment. A single mass-flow controller maintained the CO_2 set point to within 2% for the duration of each trial. Two trials were maintained at 400 μmol mol^{-1} and two at 1,200 μmol mol^{-1}. The 1,200 μmol mol^{-1} CO_2 level was used as the elevated CO_2 treatment because photorespiration would be considerably reduced and respiration would not be limited by carbohydrate supply in those trials. The elevation of Utah State University is about 1,500 m above sea level.

Manual adjustment of pH on a daily basis resulted in a one pH unit day-to-day range. The pKa of carbonate is 6.2, which means that 50% of the carbon dissolved in the water is in the carbonate form and 50% is CO_2. Due to the limitations of our pH control method, a one pH unit range during the day has the potential to cause significant fluxes in and out of the nutrient solution. For this reason, the pH of the hydroponic solution was maintained between 4 and 5, which forces between 90 and 99% of the CO_2 out of solution.

Two trials were conducted with constant day/night temperatures of 21.5, 25, 30,

32.5, and 35C, and two trials were conducted with different day/night temperatures of 21/18, 26/21, 29/24, 32/27, and 35/30C. Temperatures of the root zone solution were the same as the average daily temperature. Temperatures were measured with aspirated type E (0.25mm diameter) thermocouples. Air temperature control was within ±0.2C of the set point, and root temperatures were within ±0.5C of the set point. Relative humidity was controlled to between 60 and 80%. PPF was provided by water-filtered, high-pressure-sodium lamps that provided 600 μmol m^{-2} s^{-1} (±5% between chamber variability) for a 16-h photoperiod. As the canopy grew taller, reflective material wrapped around each chamber was raised to the top of the canopy to reduce side lighting. Tipburn (Ca deficiency localized at the meristem) was observed in all trials, but was not severe because of the intermediate PPF used in these trials as well as the selection of 'Grand Rapids' cultivar (Appendix A).

Ground cover was measured daily until 100% cover using a digital camera. Images were processed with software to discriminate between leaf color and ground cover (Klassen et al., In press). Pixels that were determined to be leaf were counted, and this number was divided by the total number of pixels for the ground area. Resulting canopy cover errors were corrected as described by Klassen et al. (In press). Days to canopy closure, therefore, is the number of days from transplanting until 100% ground cover.

Chlorophyll content was estimated with a portable clamp-on chlorophyll meter (Minolta Model SPAD-502). The SPAD values were converted to chlorophyll concentration (mg chlorophyll m^{-2} leaf) using the equation described in Monje and Bugbee (1992).

Calculations

Carbon use efficiency is a calculated term that measures how well plants can incorporate the carbon fixed during the day into biomass gain. Using the measured CO_2 exchange rates of P_{net} (net photosynthesis, mol C m^{-2} d^{-1}) and R_n (night-time respiration, mol C m^{-2} night^{-1}), daily carbon gain (DCG) can be calculated as:

$$DCG = P_{net} - R_n.$$

P_{gross} is a calculated term that reflects the net C fixed (P_{net}) and the amount of C that is simultaneously being respired. Because day-time respiration (R_d) can not be measured directly, P_{gross} is expressed as the sum of P_{net} and some percentage of R_n. Some studies found that R_d in leaves can be higher during the day due to their higher daytime carbohydrate content (Azcón-Bieto et al.,1983; Azcón-Bieto and Osmond, 1983). Other studies indicated that daytime R_d is lower due to light inhibition of respiration (Sharp et al., 1984). Monje and Bugbee (1996) found that root respiration, at a constant temperature, is increased in the day presumably due to increased carbohydrate supply. The common approach is to assume that the rate of R_d and R_n (μmol m$_{ground}^{-2}$ s^{-1}) are equal when temperatures are constant. In a 12-h photoperiod, R_d (mol m$_{ground}^{-2}$ d^{-1}) then equals R_n. In a 16-h light / 8-h dark photoperiod, $R_d = R_n * 2$. In these equations, respiration assumes a positive value (i.e., mass respired). P_{gross} (mol C m$_{ground}^{-2}$ d^{-1}) can, therefore, be calculated as:

$$P_{gross} = P_{net} + R_d$$

CUE is the ratio of carbon gained per day to total carbon fixed, or:

$$CUE = DCG / P_{gross}.$$

Sensitivity analysis of CUE to changes in R_d to calculate P_{gross} indicated that R_d can change as much as 50% higher or lower, which can result in changes of CUE from 0.65 to 0.575, or from 0.65 to 0.73, respectively. Therefore, the assumption of constant day and night respiration rate has little impact on the calculated value of CUE. Furthermore, changes in CUE relative to the control are more important than absolute changes to CUE with shade application. To calculate the R_d from R_n to determine P_{gross}, Q_{10} values of 1.2 were used for total respiration, as reported for lettuce in Chapter 2.

Statistical Analysis

A randomized block design was used with five treatments in each of two blocks, giving two replicates at each temperature. Occasionally, temperature control at the low or high treatments could not be duplicated, so linear regression analysis was used to treat temperature as a continuous variable rather than as discrete treatments. Temperature control of the root zone was lost occasionally, which resulted in at least a 7C difference between the root zone and shoot temperature. In those cases, the data were discarded. Average PPF absorption was analyzed with ANOVA and Tukey's comparison of means to find treatment differences in average amount of light absorbed.

Results

400 μmol mol⁻¹ CO₂, Constant Temperature

Data from both 32.5C treatment and one of the 35C treatments were discarded due

to lack of control of root zone temperatures. With the remaining chambers, growth rates clearly differed among the different temperature treatments (Figure 3-1A). The 21.5C and 35C treatments had the lowest final photosynthetic and respiration rates per unit ground area. The highest gas exchange rates were obtained in the treatments with average daily temperatures of 30C.

Three of the determinants of yield (PPF absorption, QY, and CUE) are summarized in Figure 2. There was a strong influence of temperature on radiation capture (Figure 3-2A). The 30 and 25C treatments both reached near maximum PPF absorption on Day 21. Conversely, the 35C treatment never reached that amount of PPF absorbed. Because radiation capture is a determinate of growth, the different magnitude of ground cover contributed to the different growth rates. Averaged across the 26 days after transplanting, temperature had a significant effect on average daily PPF absorbed (F = 91.75, df = 3, P = 0.011; Figure 3-2B). Based on Tukey's comparison of means, the 35C treatment was significantly lower (P < 0.05) than the 21.5C, 25C, and 30C treatments, and the 21.5C treatment was lower than the 30C treatment.

Although the 21.5C treatment nearly reached canopy closure by the end of the trial, it still had about half the photosynthetic rate as the 25 and 30C treatments. Chlorophyll content is strongly related to temperature (Appendix A). The 21.5C treatment had chlorophyll contents between 2.6 and 7.6 mg chlorophyll m^{-2} leaf, which is extremely low. As a result, the digital imaging technique to estimate absorbed PPF probably over-estimated the amount of light a single leaf can absorb when chlorophyll content was low. Correcting for this, QY decreased significantly with warmer temperature (slope = -.0010;

F = 14.62, df = 33, P = 0.0006; Figure 3-2D), so while the plants in the cooler

temperatures had efficient photosynthesis, they may not have absorbed enough light to

have high growth rates. There was a tendency for QY to increase for all treatments near

the end of the trial (lowest significance: F = 19.99, df = 3, P = 0.021; Figure 3-2C).

Values of CUE reached about 0.55 for all temperature treatments except the 35C

treatment (Figure 3-2E). There was a significant effect of temperature on CUE (F =

118.46, df = 3, P < .001; Figure 3-2F) with the warmest treatment having a value of about

0.3 (±0.12 std dev.) during the last 11 days of the trial. The CUE of the 21.5C treatment

was significantly different from the other treatments, based on Tukey's comparison of

means (P<0.001), and the 35C treatment was lower than all other treatments. The 25C

and 30C did not differ significantly from one another (P = 0.28).

1,200 µmol mol^{-1} CO_2,
Constant Temperature

The highest growth rates occurred in the 30C and 32.5C temperature treatments, and

the lowest occurred in the 35C and 21.5C treatments (Figure 3-1B). The coolest and

warmest temperatures (21.5 and 35C, respectively) had the lowest final photosynthetic

and respiration rates per unit ground area. All final growth rates were higher than the

similar temperature treatments in the 400 µmol mol^{-1} CO_2 trial.

Temperature significantly affected radiation capture (F = 10.04, df = 5, P = 0.043;

Figure 3-3A and 3-3B). The 30C treatment reached maximum PPF absorption (about 33

mol absorbed m^{-2} d^{-1}) by Day 17, which was four days faster than that observed in the 400

µmol mol^{-1} CO_2 trial. Conversely, the 35C treatment reached a maximum of 10 mol

photons absorbed m^{-2} d^{-1}, but only differed significantly from the 32.5C treatment (P < 0.05). Averaged across the 24 days after transplanting, the 25 and 30C treatments had the highest daily PPF absorbed (Figure 3-3B).

Increasing temperature significantly increased QY (slope = 0.0024; F = 244.0, df = 63, P < 0.0001; Figure 3-3C and 3-3D). The 23C, 30C, and 35C treatments changed significantly with time (lowest F = 11.22, df = 10, P = 0.0074), but the other treatments did not (P at least 0.57). This analysis suggests that the 35C treatment was photosynthesizing efficiently, but lacked leaf area to have high photosynthetic and growth rates.

Values of CUE were eventually similar in all temperature treatments (Figure 3-3E). Temperature did not affect CUE (F = 0.72, df = 23, P = 0.41; slope = -0.0003; Figure 3-3F), and all treatments maintained an average CUE of 0.62 with a standard deviation of 0.02 during the final ten days of growth.

400 μmol mol^{-1} CO_2, Day/Night
Temperature Differential

Data from one of the 32/27C treatments were discarded due to lack of control over its root zone temperatures. With the remaining chambers, growth rates were similar in the different temperatures with the exception of the 35/30C treatment (Figure 3-1C). That treatment had the lowest final photosynthetic and respiration rates per unit ground area.

Temperature had a strong influence on radiation capture (F = 34.4, df = 4, P = 0.008; Figure 3-4A). The 32/27C treatment reached near maximum PPF absorption on

Day 21. Conversely, the 35/30C treatment never reached that amount of PPF absorbed. Averaged across the 24 days after transplanting, the 32/27C treatment was significantly higher than either the 29/24C or the 35/30 C treatment (P < 0.05; Figure 3-4B).

The QY decreased as temperature increased (slope = -0.0015, F = 47.62, df = 61, P < 0.0001; Figure 3-4D), but the 35/30C treatment never absorbed enough light to have high growth rates. Because of higher QY and lower PPF absorbed per day, the photosynthetic rates were similar in all treatments except the 35/30C treatment. There was a tendency for QY to increase for all treatments at the end of the trial (lowest F value = 5.13, df = 22, P = 0.034; Figure 3-4C).

Values of CUE reached about 0.62 for all treatments (Figure 3-4E). There was a statistically significant effect of temperature on CUE (F = 9.65, df = 61, P = 0.0029), but the slope was only -0.0016 (Figure 3-4F) with the 35/30C treatment having a value of about 0.60 (± 0.011 std. dev.) during the last seven days of the trial.

1,200 μmol mol^{-1} CO$_2$, Day/Night
Temperature Differential

Lettuce growth differed substantially in the different temperatures (Figure 3-1D). The 23/18C and 35/30C treatments had the lowest final photosynthetic and respiration rates per unit ground area. The highest gas exchange rates were obtained in the treatments with average daily temperatures of 27 or 30C.

Temperature significantly influenced radiation capture (F = 45.03, df = 4, P = 0.001; Figure 3-5A). The 29/24C and 32/27C treatments reached near maximum PPF absorption by Day 16. Conversely, the 35/30C treatment never reached that amount of

PPF absorbed. The different magnitudes of ground cover likely contributed to the different growth rates. Averaged across the 24 days after transplanting, the 29/24C and 32/27C treatments were significantly greater than the 35/30C and 23/18C treatments (P < 0.05; Figure 3-5B).

The 23/18C and 35/30C treatments had similar photosynthetic rates per unit ground area (Figure 3-1D), but had different rates of canopy closure with the 23/18C treatment reaching maximum absorption after 22 days and the 35/30C treatment not reaching that level. Canopy QY was higher with temperature as observed in the other high CO_2 trial (slope = 0.0015; F = 86.68, df = 28, P < 0.0001; Figure 3-5D). Canopy QY changed significantly through time for the 29/24C and the 32/27C treatments (highest F value = 6.28, df = 20, P = 0.021; Figure 3-5C), but not for the other treatments (highest F value = 0.94, df = 17, P = 0.35).

Values of CUE were stable and similar in all treatments after Day 9, and all temperature treatments had a similar CUE of 0.64 (±0.017 std. dev.) after that (Figure 3-5E). Temperature did not influence CUE (F = 1.85, df = 108, P = 0.18; slope = 0.0005; Figure 3-5F).

Discussion

Surprisingly little information is available concerning the effect of day and night-time temperature on carbon gain. McCree and Amthor (1982) reported that improved carbon balanced led to increased growth rate of plants (about 15%) when grown at a constant 20C compared to growth at 30/10C day/night. This was attributed to excessive

dark respiration during the day and only slightly reduced night-time respiration. In our studies, carbon gain was not greater with cool night-time temperature and warm day-time temperatures. This is similar to the results of Chapter 2 where daily carbon gain was not affected by either cooler or warmer night-time temperatures.

Improved PPF absorption coincided with greater final mass and absolute growth rates in this study. Temperature played a significant role in determining the rates of leaf expansion and emergence (deduced from digital images of ground cover through time), and as a consequence, the amount of light captured.

The high CO_2 treatments hastened canopy closure and maximum light interception by about three days. However, these plants were grown hydroponically, so it is unlikely the plants experienced water stress. Therefore, partial stomatal closure due to high CO_2 was not believed to lead to better water relations and improved leaf expansion. High CO_2 led to faster growth rates perhaps because they were not substrate limited for growth. This may have been the primary reason for hastened canopy closure and improved light interception.

Once light was intercepted, QY was higher in elevated CO_2 and was lower in ambient CO_2, as expected. The values of QY were about the same as those calculated from Wheeler et al. (1994) for groups of lettuce plants grown in 1,000 μmol mol^{-1} CO_2 in lower light. Photorespiration should increase with an increase of temperature from 20C to 35C in ambient CO_2, causing QY to decrease from about 0.065 to about 0.057 (Farquhar et al., 1980; Long, 1991). In the ambient CO_2 trials in this study, QY decreased from 0.04 mol C fixed per mol photons absorbed to about 0.03 mol C fixed per mol

photons absorbed at the warmest temperature, which is about the same magnitude as that

predicted from the model of Farquhar et al. (1980). Few studies have investigated

quantum yield on a whole plant or whole canopy basis in elevated CO_2 across a range of

temperatures. Photosynthetic models predict that while the oxygenation reaction of

Rubsico should increase with temperature, electron transport would increase and peak

about 30 to 35C (Farquhar et al., 1980; Jordan and Ogren, 1984). If elevated CO_2

minimizes photorespiration, then the QY should increase with temperature as electron

transport becomes the limiting factor of photosynthesis. The magnitude of increase in

QY for plants grown in high CO_2 in high temperatures in this study confirms the

biochemical models on a whole plant basis, even at relatively high light. The observed

increase was large enough that in spite of large differences in moles of photons absorbed

between the warmest and coolest treatments in high CO_2, their photosynthetic rates were

similar at the end of the trial.

Carbon use efficiency has been modeled to be a function of growth conversion

efficiency, the maintenance coefficient, and relative growth rate (RGR) (Thornley and

Johnson, 2000). According to this model, CUE should decrease as RGR decreases.

However, because the model uses a hyperbolic-type equation similar to the Michaelis-

Menton enzyme kinetics equation, RGR can vary greatly without a substantial change in

CUE. Indeed, RGR varied considerably across all the treatments for the high-CO_2

treatments, yet CUE was about the same. It is possible that CUE was the same across all

temperatures in the high CO_2 treatment because the plants acclimated by altering the

growth conversion efficiency or the maintenance coefficient with high temperatures. If

this were the case, why did the ambient-CO_2 treatment not adjust, resulting in constant CUE? CUE represents the balance between carbohydrate supply and demand. High CO_2 grown plants are known to accumulate sugars and starch relative to those grown under ambient CO_2, and because of this, demand for carbohydrate in growth and maintenance may have never exceeded carbohydrate supply. With increasing temperature, maintenance respiration probably increased because protein turnover, ion leakage, and membrane repair likely increased. Exactly how much maintenance respiration increased is unclear. Given the length of time these plants were exposed to their respective temperatures and the range of temperatures in which these canopies grew, the Q_{10} for maintenance respiration is probably between 1.2 and 1.5. These values suggest that demand for carbohydrates may have varied for plants in the different temperatures, and resulted in different values of CUE.

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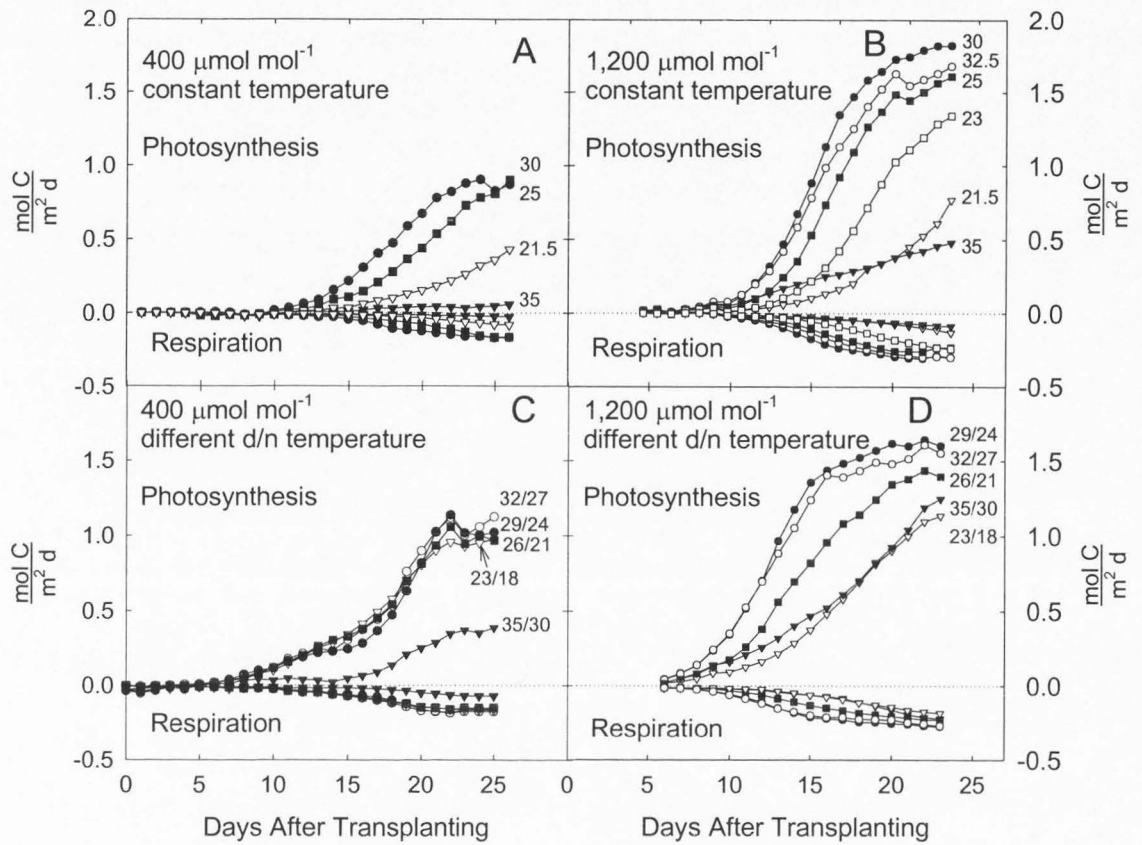
Figure 3-1. Photosynthesis and respiration rates for all temperature and CO_2 trials.
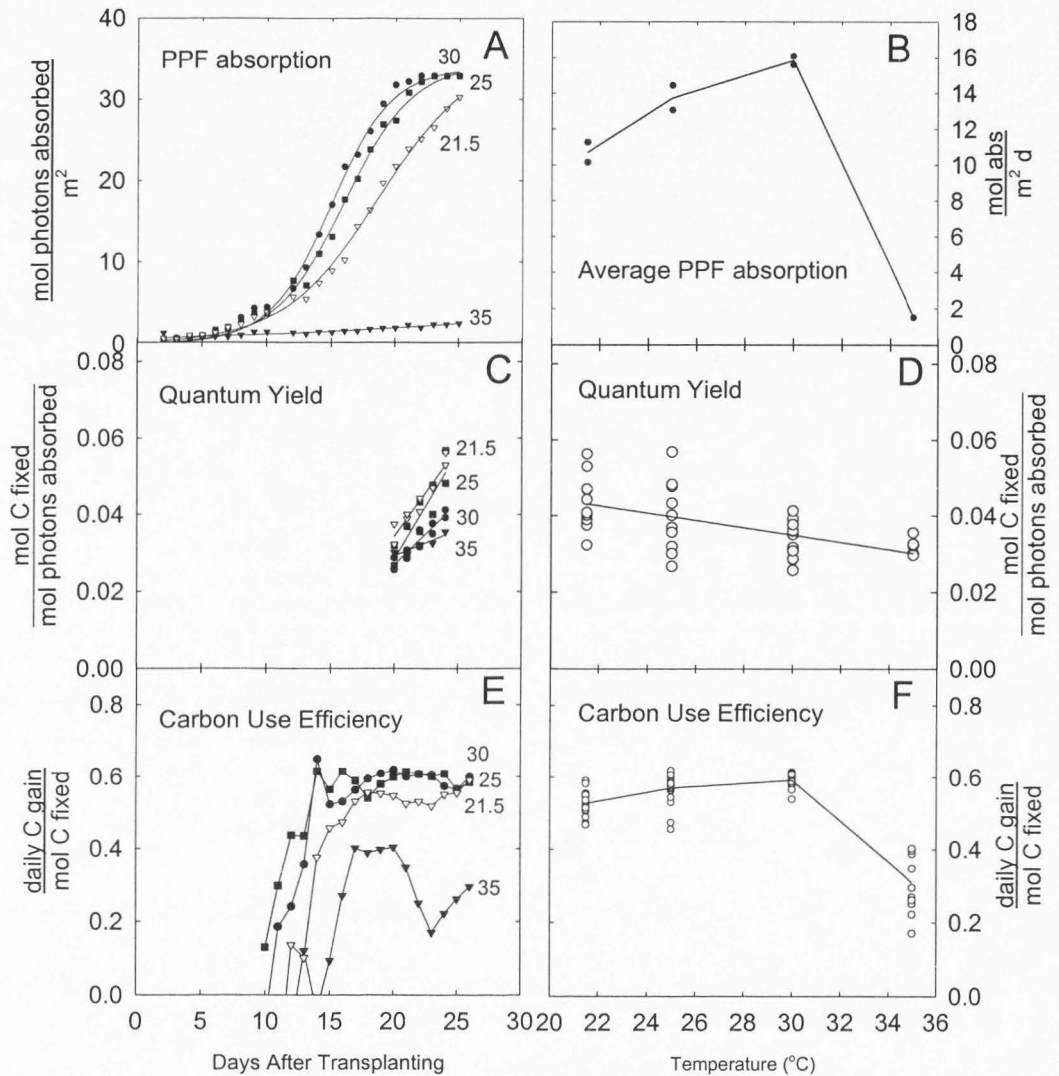
Figure 3-2. Three determinants of yield expressed on both a time and a temperature basis for lettuce canopies grown in 400 μmol mol^{-1} CO_2 with constant temperatures.
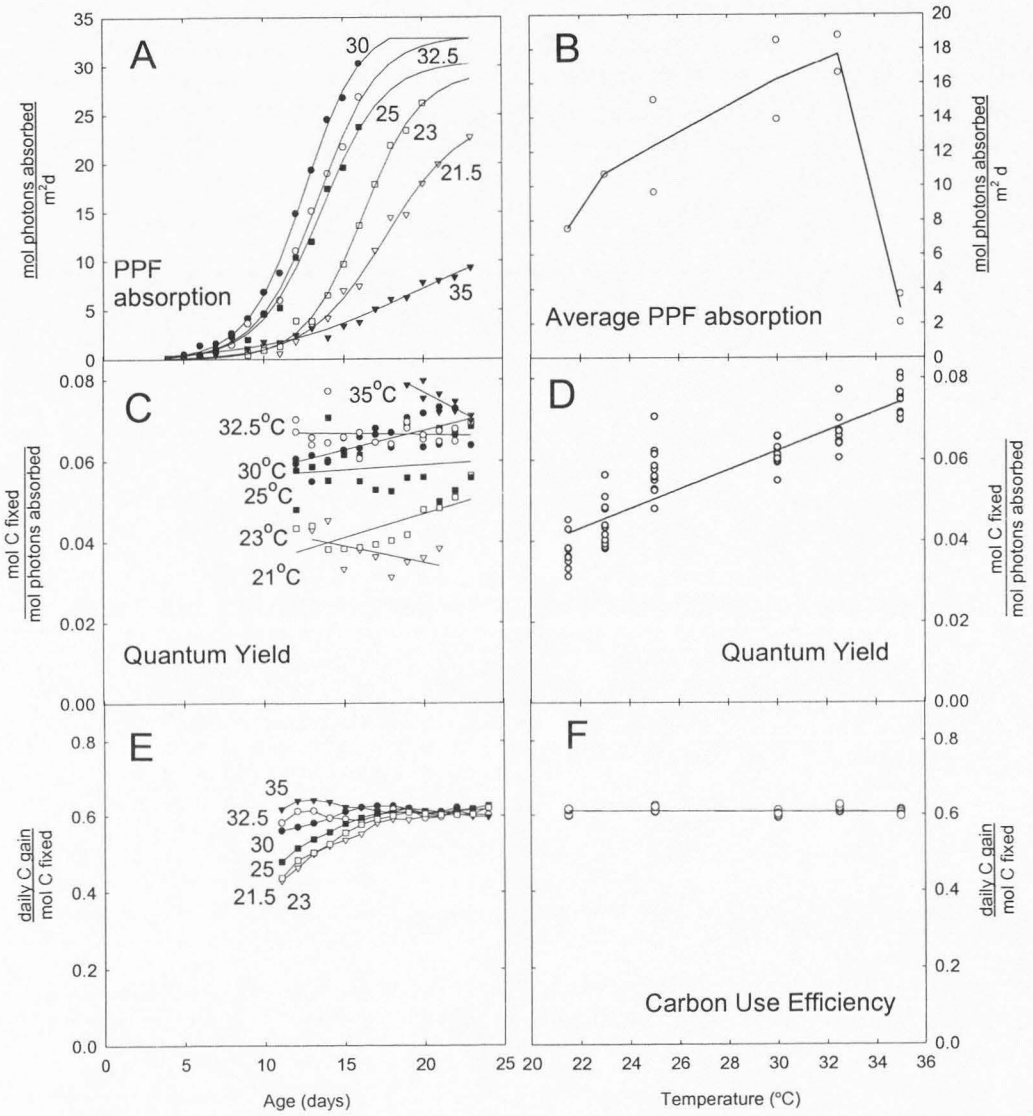
Figure 3-3. Three determinants of yield expressed on both a time and a temperature basis for lettuce canopies grown in 1,200 μmol mol^{-1} CO_2 with constant temperatures.
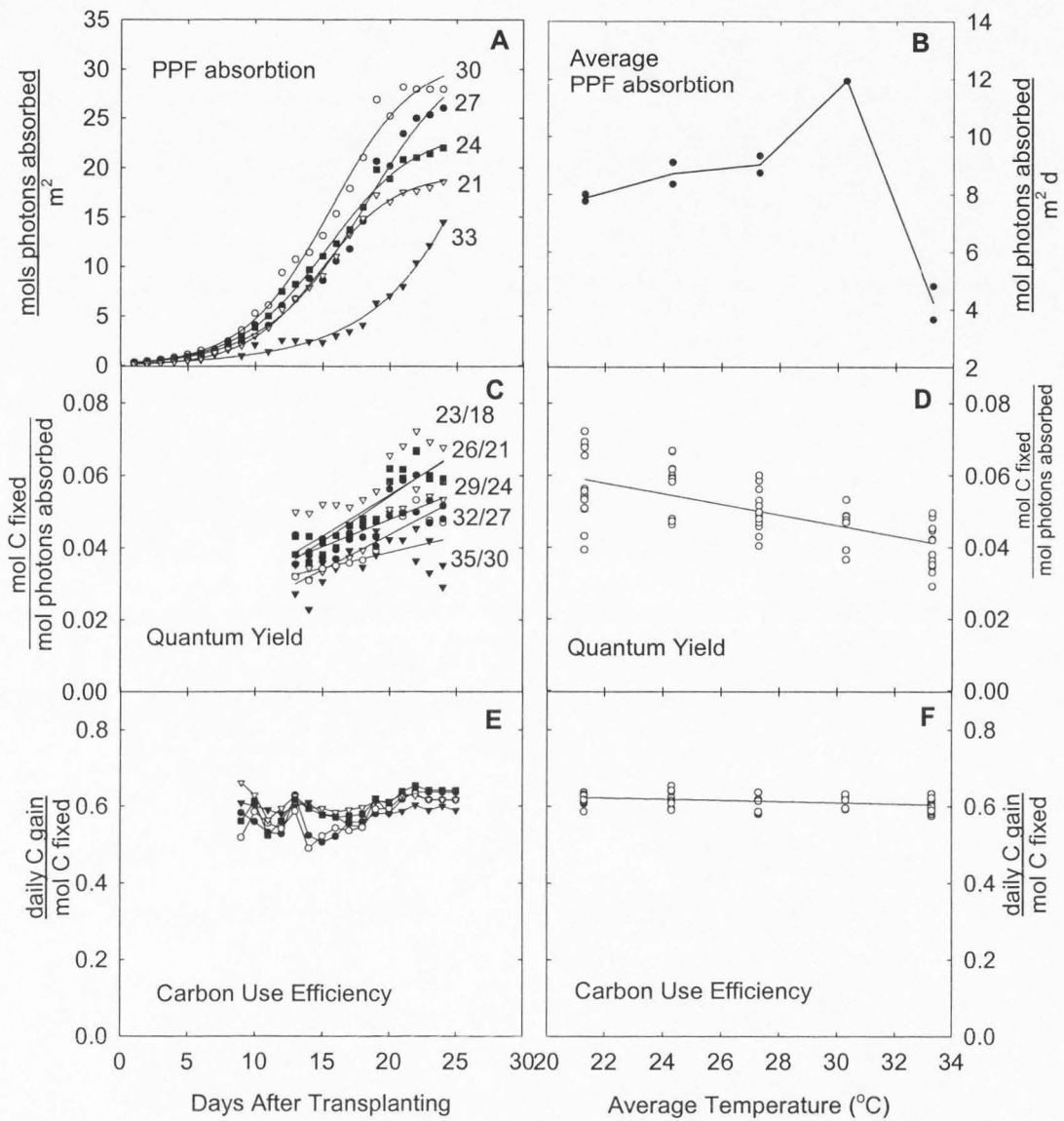
Figure 3-4. Three determinants of yield expressed on both a time and a temperature basis for lettuce canopies grown in 400 µmol mol⁻¹ CO_2 with a 5C difference in day/night temperatures.
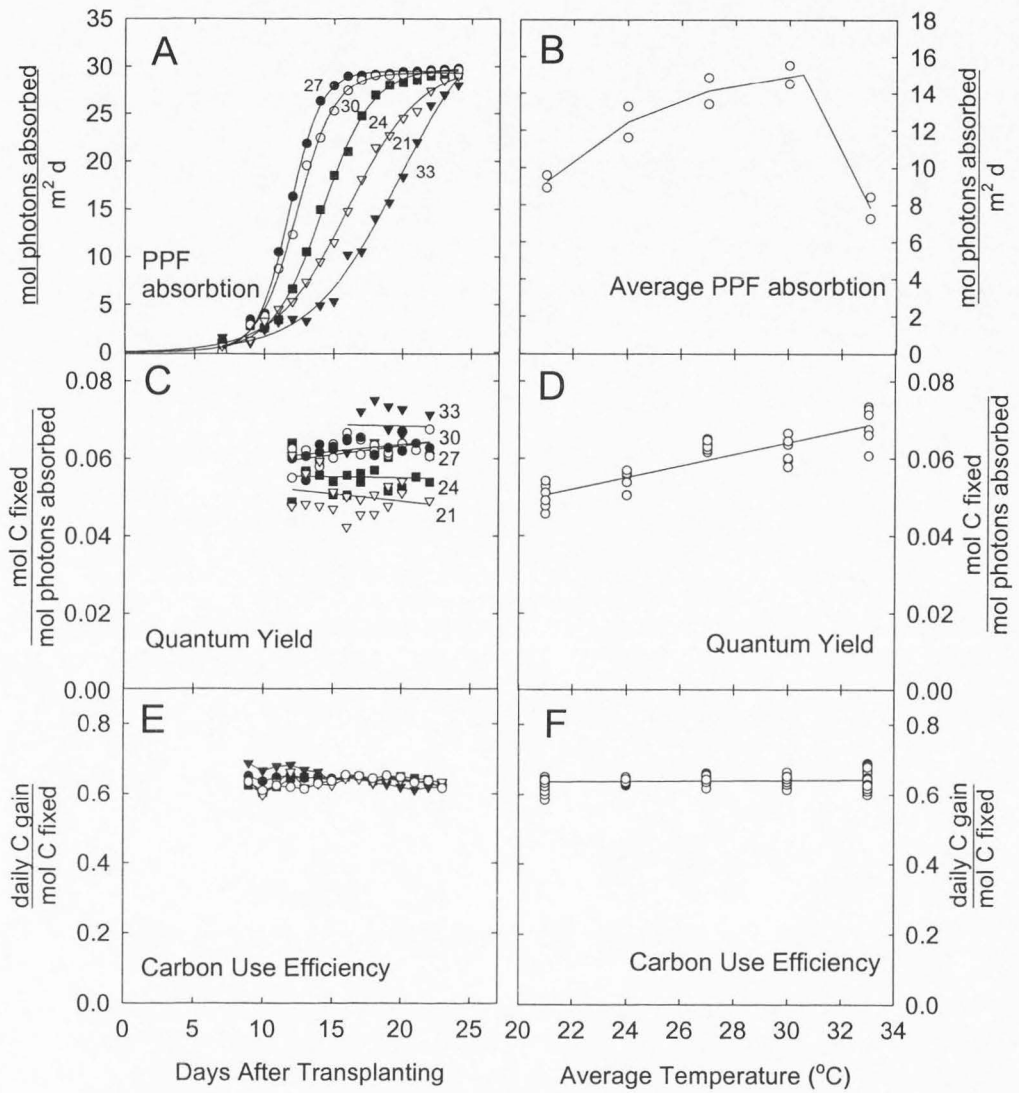
Figure 3-5. Three determinants of yield expressed on both a time and a temperature basis for lettuce canopies grown in 1,200 µmol mol^{-1} CO$_2$ with a 5C difference in day/night temperatures.

CHAPTER 4

ACCLIMATION TO SHADE: PHOTOSYNTHESIS, RESPIRATION,

AND CARBON USE EFFICIENCY[3]

Abstract

Photosynthesis, respiration, and the balance between the two change in response to

the environment. Surprisingly few studies have examined how quickly and how

completely plants acclimate to the environment on a whole plant or canopy basis.

Canopies of tomato (*Lycopersicon esculentum* Mill.) and lettuce (*Latuca sativa* L.) were

subjected to a range of shade after canopy closure in a growth chamber. Photosynthesis,

respiration, and carbon use efficiency (ratio of carbon gain to carbon fixed) were

measured for up to 18 days after shade was applied. Canopies of lettuce and tomato did

not immediately acclimate, in contrast to popular growth models, and the two species

acclimated in different ways. Lettuce grown in 80% shade never completely acclimated,

whereas tomato acclimated after 12 days. Both species had more efficient photosynthesis

(higher canopy quantum yield) after shade application. Tomato and lettuce were able to

adjust relative growth rate, C partitioning, or growth or maintenance respiration to

maintain carbon use efficiency, but did so at a much slower rate than currently modeled.

Introduction

Daily light levels change by an order of magnitude during the growing season, but

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[3]Coauthored by Bruce Bugbee.

little is known about how quickly and completely photosynthesis and respiration respond to shade. Respiration is considered to be both supply (carbohydrate content) and demand dependent (sensitive to temperature and type of biomass) (Amthor, 1989). While the relationship between respiration and temperature is generally well described, a strong correlation between respiration and carbohydrate content has been established in several studies (Azcón-Bieto and Osmond, 1983; Moser et al., 1982; Azcón-Bieto et al., 1983). The data are, however, typically based on short-term measurements made on plant parts rather than whole plants or plant communities (Geiger et al., 1998; Hill and ap Rees, 1995). For example, Sims and Pearcy (1991) investigated leaf CO_2 gas-exchange upon transfer from low to high and from high to low light and found that leaf respiration reached steady state levels within one week. Other studies reported no relationship between the amount of light a leaf receives and its subsequent respiration (Groninger et al., 1996). Only a few studies have investigated whole-plant or whole-community gas exchange (Percival et al., 1998; Wheeler et al., 1993), and the relationship between respiration and carbohydrate status on a whole-plant basis was not clear.

High photosynthetic rates increase carbohydrate content in leaves. For example, Ayari et al. (2000) indicated that leaf concentrations of starch, sucrose, and hexose varied with amount of light, photosynthetic rates, and leaf position. Carbohydrate content also can vary within a leaf in a wheat (*Triticum aestivum* L.) canopy with vertical leaves as a function of incident photosynthetic photon flux (PPF) and position in a canopy (Smart et al., 1994). Because carbohydrate content varies within a plant and within a leaf, respiration rates within different plant parts also may vary. Even if a representative leaf

could be chosen, the leaf is not likely to represent the respiration of other plant parts. Monje and Bugbee (1996) saw increased root respiration during the day, probably due to increased carbohydrate supply to the roots. Meristems are also likely to have much higher respiration rates because they are a strong sink for carbohydrates in a growing plant. This underscores the importance of measuring whole plant gas-exchange when attempting to determine the influence of PPF on plant growth rather than relying on single leaf measurements.

Many studies have investigated the long-term effects of shade on plant growth and yield (Sanchez et al., 1989; Barbour et al., 1994; Egli, 1997). It is generally accepted that growth is reduced in nearly direct proportion to the decrease in PPF. However, there is a poor understanding of how whole plant respiration, photosynthesis, and the balance between the two acclimate in response to shade. The models that permit imposition of shade indicate immediate acclimation and little difference in response among species (J. Cavazzoni, personal communication).

In this study, we used a 10-chamber gas-exchange system that permitted continuous monitoring of the growth of groups of whole plants before and after shade. I hypothesized that application of shade would immediately reduce photosynthesis, but respiration would respond more slowly because of stored carbohydrates. To test the effects of carbohydrate status, lettuce (*Latuca sativa* L.), a low starch accumulator (Forney and Austin, 1988) was compared to tomato *(Lycopersicon esculentum* Mill.), a starch accumulator (Hocking and Steer, 1994). It was hypothesized that species differences would exist in both response to shade and rate of acclimation to shade.

Materials and Methods

Experimental Design

Two trials were conducted, each with a different species. Lettuce (*Latuca sativa* L. cv. 'Grand Rapids') and tomato (*Lycopersicon esculentum* Mill. cv. 'Micro-Tina') were germinated and transplanted after five days into a 10-chamber gas-exchange system previously described by van Iersel and Bugbee (2000). Lettuce was grown at a density of 106 plants m^{-2}, and tomato was grown at a density of 88 plants m^{-2}. For each trial, shade treatments were arranged in a randomized incomplete block design and analyzed using linear regression. Both blocks contained a control (zero shade) and high shade (about 80% reduction in PPF) treatment, but the amount of shade for the other three canopies in each block was not identical. ANOVA was performed on data to test for significant differences between chambers before treatments were applied. Slopes of lines were compared to see if their slopes were equal using the test statistic ((slope a - slope b) - 0)/(variance of slope a) = t $_{(degrees\ of\ freedom\ of\ slope\ a)}$.

Plant Growth Environment

Each chamber in the gas-exchange system was 0.5 x 0.4 x 0.9 m (L x W x H) and fully enclosed a hydroponic tub. Both crops were grown at constant 25C day/night temperature including the roots. Chamber temperature was controlled to within ±0.2C of set point, and CO_2 was controlled to within ±2% of a set point of 1,200 µmol mol^{-1}. Elevated CO_2 was used to ensure that photosynthesis would be limited by light rather than CO_2. Root-zone temperature was maintained by activating flexible heat-stripping

wrapped around the outside of the hydroponic tubs when the temperature fell below the set point. Hydroponic solution was bubbled with the same CO_2-enriched air as that used in the canopy. The CO_2-gas-exchange of each of 10 different whole canopies was monitored once every 10 minutes throughout the trial.

The manual adjustment of pH on a daily basis resulted in a 1 pH unit day-to-day range. The pKa of carbonate is 6.2, which means that 50% of the carbon dissolved in the water is in the carbonate form and 50% is CO_2. Due to the limitations of our pH control method, a 1 pH unit range during the course of the day has the potential to cause significant fluxes in and out of the nutrient solution. For this reason, the pH of the hydroponic solution was maintained between 4 and 5, which forces between 90 to 99% of the CO_2 out of solution. Relative humidity was maintained between 60 and 85% for the duration of the trials. The photosynthetic photon flux (PPF) was provided by water-filtered HPS lamps that provided a PPF of 600 μmol m^{-2} s^{-1} ($\pm$5%) for lettuce and 650 μmol m^{-2} s^{-1} ($\pm$5%) for tomato. Both trials were run using a 16-h photoperiod to provide 35.6 mol photons per m^2 per day for lettuce and 37.4 mol photons per m^2 per day for tomato.

Shade was applied after canopies closed, which occurred about 16 days after transplanting for lettuce and 20 days after transplanting for tomato. Shade was applied using a variable amount of neutral-density window screening (10-mesh) postitioned on the top of the chambers that reduced PPF by 50% with each complete layer of window screening. Less than 50% shade was obtained using smaller pieces of screening that made incomplete layers of screen. PPF was measured twice weekly with a line-quantum

sensor (Model LQSV-ELEC, Apogee Instruments, Inc., Logan, UT) that averaged PPF

across the top of the canopy. Shade cloth was adjusted twice weekly to maintain shade to

original shade level. A range of shade from 0 to 80% shading was obtained in each trial.

Shade continued until a typical cropping duration for lettuce, which was 9 days after

shade application. Shade continued for tomatoes for 18 days after canopy closure.

Reflective material was wrapped around each chamber and was adjusted daily to the top

of the canopy to minimize side lighting. All days are after transplanting.

Calculations

Carbon use efficiency (CUE) is a calculated term that measures the amount of

carbon incorporated into the plants divided by the total amount of carbon fixed in

photosynthesis. Essentially, it is a term describing how well plants can incorporate the

carbon fixed during the day into biomass gain and can be calculated as:

$$CUE = DCG / P_{gross}$$

where P_{gross} is gross photosynthesis and DCG is daily carbon gain. Using the measured

CO_2 exchange rates of P_{net} (net photosynthesis, mol C m^{-2} d^{-1}) and R_n (night-time

respiration, mol C m^{-2} night^{-1}); night is the night period following the photosynthesis

period, which together make up one 24-h period), daily carbon gain (DCG) can be

calculated as:

$$DCG = P_{net} - R_n$$

where P_{gross} is a calculated term that incorporates both the net C fixed (P_{net}) and the C that

is simultaneously being respired. Because day-time respiration (R_d) can not be measured

directly, P_{gross} is calculated as the sum of P_{net} and some percentage of night-time

respiration rate. Previous studies have indicated that R_d increases during the day in intact

plants due to higher carbohydrate content (Azcón-Bieto and Osmond, 1983), or can be

lower due to some type of light-inhibition of respiration in leaves (Atkin et al., 2000;

Sharp et al., 1984). Monje and Bugbee (1996) found that root respiration, at a constant

temperature, is increased in the day presumably due to increased carbohydrate supply.

The common approach for whole plants is to assume that the rate of R_d and R_n (μmol

m_{ground}^{-2} s^{-1}) are equal when temperatures are constant. In a 12-h photoperiod, R_d (mol

m_{ground}^{-2} d^{-1}) then equals R_n. In a 16-h light/8-h dark photoperiod, $R_d = R_n * 2$. In these

equations, respiration assumes a positive value (i.e., mass respired). P_{gross} can, therefore,

be calculated as:

$$P_{gross} = P_{net} + R_d$$

Sensitivity analysis of CUE to the assumption of R_d to calculate P_{gross} indicates that R_d can

change by as much as 50% higher or lower than R_n and change CUE by only 0.08 or 12%

(assuming a typical CUE of 0.65). Therefore, the assumption of constant day and night

respiration rate has little impact on the calculated value of CUE. Small changes in CUE

can be important on a day-to-day basis, but relative changes are more important than

absolute values. Furthermore, changes in CUE *relative* to the control are more important

than *absolute* changes to CUE upon shade application.

After treatments were imposed, data were expressed as a percent of their initial

value, then normalized to the control in the following manner:

$$(\text{Posttreatment}_a \ \text{day}_b \ / \ \text{Pretreatment}_a \ \text{value}) \div$$

$$(\text{Posttreatment}_{control} \ \text{day}_b \ / \ \text{pretreatment value}_{control})$$

where posttreatment$_a$ indicates the posttreatment value of parameter a (i.e., CUE, P$_{net}$, and

R$_n$), day$_b$ indicates the day after treatment, pretreatment$_a$ value is the value of the

parameter of interest on the day before treatments began, posttreatment$_{control}$ day$_b$ is the

posttreatment value of the parameter of interest on the same posttreatment day, and

pretreatment value$_{control}$ is the pretreatment value of the parameter of interest the day

before treatments began. By doing this transformation, effects of shade on a given

canopy can be assessed in the numerator, and the effects relative to the control and natural

development can be assessed in the denominator.

Quantum yield (QY) was calculated for each canopy after canopy closure. Incident

PPF was measured and 95% of that was assumed to be absorbed. The P$_{gross}$ for the day

period (mol C m^{-2} d^{-1}) was then divided by total photons absorbed (mol photons

m^{-2} d^{-1}) to give QY (mol C fixed per mol photons absorbed).

Results

Lettuce

Canopy gas exchange did not differ significantly across canopies during the 16 days

of growth prior to shade treatment in the lettuce trial (P = 0.985, Figure 4-1A). Values of

P$_{net}$ decreased immediately after applying the shade treatments with a 76% decrease

occurring in PPF of 150 μmol m^{-2} s^{-1}. Values of P$_{net}$ increased from initial post-shade

values in all treatments by 0.25 mol C m^{-2} d^{-1}. The relative increase in the control (high-light) was about 12%, whereas the relative increase in the low-light treatment was 75% (Figure 4-2A). Night-time respiration also decreased by 0.11% for each μmol m^{-2} s^{-1} decrease in PPF with a reduction of about 50% when PPF was reduced by 450 μmol m^{-2} s^{-1} (Figure 4-1A). Respiration did not change after the initial posttreatment reduction.

Carbon use efficiency (CUE) was similar before treatments were applied (P = 0.152, Figure 4-1C). During the three days before shade was applied, the CUE averaged 0.62 with a standard deviation of less than 0.012. These differences are likely due to small differences in the rate of canopy fill, PPF absorption, and side-lighting, and were not consistent among canopies. With shade application, the CUE sharply decreased in proportion to decreases in PPF. The largest decrease was in the 150 μmol m^{-2} s^{-1} treatment, which declined to a CUE of about 0.4. The next day, CUE began to increase with subsequent days exhibiting smaller increases as CUE approached pretreatment values. Beyond about 3 days, differences among treatments were not as obvious so data were normalized to pretreatment levels and expressed as a percent of control (Figure 4-3A). Values of CUE dropped to about 50% of control values at the lowest PPF, but gradually recovered to near pretreatment levels. There was a significant difference in the temperature sensitivity of the relative CUE on Day 2 and Day 3 (t = 3.51, df = 8, P < 0.005), indicating some recovery after the second day. There was no additional recovery after the third day. The trial ended after nine days of shade treatment. Although decreases in total root and shoot mass were detected, no change in the percent of root mass occurred (Figure 4-5A and 4-5B).

Pretreatment QY averaged 0.048 mol C fixed per mol photons absorbed (Figure 4-4A). QY improved on the initial day of shade with decreasing PPF ($F = 13.74$, $df = 9$, $P = 0.006$). Subsequent days resulted in significant increases in QY with lower light (lowest $t = 2.54$, $P < 0.025$) until Day 3 when no further changes occurred. A maximum QY of 0.076 was measured at the lowest PPF.

Tomato

Tomato canopy closure occurred on Day 18 and shade was applied two days later. Gas exchange did not differ significantly among canopies before shade application ($P = 1.0$, Figure 4-1B). Immediately after shade was applied, P_{net} and R_n decreased with lower PPF. P_{net} declined 75% in the 190 μmol m^{-2} s^{-1} treatment. P_{net} slowly increased after shade was applied, as was seen in the lettuce trial (Figure 4-2B). P_{net} increased proportionally more in the lowest light treatments up to more than a doubling of initial shade P_{net} rates.

Values of CUE did not differ significantly prior to shade application ($P = 0.838$) and averaged 0.64 with a standard deviation of 0.02. As for lettuce, CUE was normalized to pretreatment levels and standardized as a percentage of the control (Figure 4-3B). The day after shade application, CUE was nearly 0 in the 190 μmol m^{-2} s^{-1} treatment, indicating that the low-light canopy neither gained nor lost mass during the initial 24-h of shade. Most of the recovery occurred after only 2 days, with the lowest PPF recovering to nearly 80% of pretreatment and control values. Subsequent days resulted in varied incremental increases in CUE (comparing Day 2 and Day 12, $t = 5.66$, $df = 8$, $P < 0.001$);

after 12 days, all canopies reached control and pretreatment values.

Shade treatments were continued for 18 days. Flowering occurred on Day 27 so the treatment period included both the end of the vegetative phase and the beginning of the reproductive phase. Plants set fruit in all treatments, and correlations between fruit mass and PPF were statistically significant (Figure 4-5A). Shoot and root mass decreased with increasing shade, but did so in a manner that changed the proportion of root mass at harvest. The percentage of roots in lower light was roughly half of the high-light controls (Figure 4-5B).

The pretreatment QY averaged about 0.05 mol C fixed per mol photons absorbed (Figure 4-2B). There was no significant increase in QY for any of the treatments until after the second day of shading (Day 3 $F = 22.57$, df = 9, P = 0.0014). Values of QY increased significantly between Day 3 and Day 12 ($t = 5.1$, df = 8, P < 0.0005), indicating photosynthetic acclimation to reduced PPF. A maximum QY of about 0.08 was measured at the lowest PPF after Day 12.

Discussion

In our studies, shade was applied during the vegetative phase. For tomato, the shade treatment extended into flowering and fruit set. Zhao and Oosterhuis (1998) suggested that plants can compensate for early shade stress if the stress is removed before anthesis by making broader, thinner leaves and increasing chlorophyll content. However, the ability of a plant to compensate appears to be species dependent (Mbewe and Hunter, 1986).

Lower light caused C allocation to shift from roots to shoots in tomato, but not in lettuce. While most species tend to follow a general allocation pattern, they can shift this pattern in response to the environment (Enquist and Niklas, 2002). Stoller and Myers (1989) reported that some weed species were better able to acclimate to shade stress by shifting their root:shoot ratio in favor of more shoots. Weed species that were less able to make this switch were poorer competitors and less able to adapt to shade. Lower light usually reduces transpiration rate, so fewer roots are needed to supply the water necessary for transpiration. Tomato may have shifted C allocation from roots as a way to grow more leaf area, and tomato may have a better ability to adjust than lettuce.

Reduced PPF immediately decreased P_{net} in both species. For both species, we observed a near 1:1 ratio in amount of shade in relation to reduced P_{net}. This ratio is analogous to *canopy* light response curves (Bugbee, 1992; Westgate, 1999), but differs from *leaf* light response curves (Bunce, 1991). Canopies tend to light saturate at much higher PPF than single leaves due to multiple leaf layers causing mutual shading. Net photosynthesis increased proportionately more in the low light treatment for both species. Some of this shift may have resulted from greater light absorption, but the change is likely to be small after canopy closure and should have been accounted for with standardization of the data relative to the control.

Thornley and Johnson (1990) described CUE as a function of relative growth rate (RGR = mol new C per mol existing C per time), maintenance respiration (r_m), and growth respiration (r_g) in the following manner:

$$1 / CUE = 1 + r_g + r_m * 1 / RGR$$

Therefore, a change in CUE should be the result of a change in RGR, r_g, or r_m. It is difficult to separate growth and maintenance respiration reliably and non-destructively, but RGR can easily be measured with gas exchange. Reduced PPF reduced RGR in both species so a change in CUE can be explained simply by reduced RGR. However, RGR was lower in the low-light tomato canopy than the high-light control canopy, yet had the same CUE. This suggests that either r_g or r_m acclimated so that CUE returned to pretreatment levels. The Thornley and Johnson (1990) approach assumes steady-state growing conditions, and in our study, PPF was changed before a new steady-state condition was reached. Mobilization of C may have occurred in response to shade, making it difficult to determine the extent to which r_g or r_m may have acclimated to shade. The differences in acclimation of CUE between lettuce and tomato suggest that species may have different capacities to acclimate r_g and r_m under stress. The differences in acclimation may also be partly due to differences in C allocation from the roots to shoots. Because root growth was reduced proportionally more in tomatoes than lettuce, reducing root growth may have reduced the maintenance cost for tomato.

If carbohydrate supply was equal to demand for both growth and maintenance processes, the CUE should have been unaffected by shade. That is, a reduction in photosynthesis should have resulted in an equal reduction in respiration, and, therefore, CUE should have remained constant. In this study, shade decreased CUE for both species, indicating respiration rates are dependent on carbohydrate concentration, as other studies have shown (Azcón-Bieto and Osmond, 1983; Moser et al., 1982; Azcón-Bieto et al., 1983). The differences in CUE response between species (starch accumulator vs. a

non-accumulator) suggests that plants can access carbon that was fixed on previous days. This is referred to in models as remobilization or redistribution. While these reserves may be used during stress, r_g and r_m may have been slow to adapt because carbohydrate was present, while RGR immediately decreased. As a result, the canopies did not gain mass with the same efficiency as before the stress. This concept was suggested by Kiniry et al. (1992) who reported reductions in the ability of maize (*Zea mays*) and sorghum (*Sorghum bicolor*) to tap stored reserves when stress occurred during the grain filling period.

Both tomato and lettuce acclimated at least partially to shade, however. Most of the change in CUE from initial shade levels to "normal" levels occurred after 3 days indicating a fast, but not instantaneous response to reduced PPF. Values of QY for lettuce increased rapidly immediately after shade application and reached steady values after three days. This indicates that not only did respiration decline with decreasing carbohydrate content to balance CUE, but that photosynthesis became more efficient. Improved efficiency may have been the result of reallocation of resources from Rubsico to light-harvesting complexes and/or the tendency to increase QY under low light. Tomato also had such a response, but QY acclimation in tomato was delayed by two days. This may be the result of starch accumulation in the leaves reducing photosynthetic efficiency (feedback inhibition) until adequate time had passed for respiration to use the carbon stores. Values of QY approached its maximum at low PPF, as is typical under these conditions (Lal and Edwards, 1995). The QY measured in the low-PPF canopies approached 0.08 mol C per mol photons, which is below the theoretical limit (0.083 to

0.111) for a C3 species (Lal and Edwards, 1995; Bjorkman, 1981).

Lettuce failed to completely readjust its CUE under extremely low PPF, suggesting some limits to low PPF tolerance in lettuce. The PPF obtained following shade application was definitely above the light compensation point for canopy photosynthesis as indicated by the positive daily carbon gains and positive CUE, but it may have been lower than was necessary to meet growth and maintenance requirements. Lettuce is commonly grown in growth chambers at about 300 to 400 μmol m^{-2} s^{-1} (Hammer et al., 1978), and canopies receiving that amount of light completely acclimated (based on CUE returning to pretreatment values) after 3 days. The failure of CUE in lettuce to completely acclimate based on CUE values under low-light conditions suggests that carbohydrate supply did not completely meet demand for growth and maintenance. Because CUE in tomato did completely acclimate by returning to pretreatment levels, both growth and maintenance requirements were apparently met either because tomato plants are inherently more efficient or because they are better able to acclimate to shade. Presumably, the mobilized carbon is initially in the form of starch (Penning de Vries et al., 1989), but in lower light (more than 50% shade) leaves may begin to adapt by converting from "sun" leaves to "shade" leaves. Shade leaves were able to increase their chlorophyll per unit area and leaf thickness when transferred from 40 μmol m^{-2} s^{-1} PPF to 1200 μmol m^{-2} s^{-1} (Kamaluddin and Grace, 1992). Although little evidence exists concerning cell wall degradation as an acclimation to shade (Kephart and Buxton, 1993), Allard et al. (1991) concluded that both anatomical and physiological processes are modified to adapt to reduced PPF.

Studies have reported values of CUE of about 0.6 for a wide range of species, stages of development, and plant sizes (Monje and Bugbee, 1998; Gifford, 1994; Gifford, 1995). While this study did not specifically address the idea of plants actively maintaining their CUE at some value, these data seem to suggest that a CUE of about 0.6 is maintained regardless of PPF. In a recent paper, Barford et al. (2001) examined carbon budgets of an entire forest and provided evidence suggesting that whole ecosystem CUE is about 0.6 as well. Amthor (1989) reported values of CUE for many species that ranged from 0.03 to 0.85, which were based on several different methods of calculating CUE.

Dewar et al. (1998) hypothesized that a constant CUE was the result of a plant averaging C mobilization rates in response to average variations in its environment. Their model was constructed assuming both growth and maintenance demands were met under such steady-state conditions, so this concept may not be applicable under extremely low light or under conditions where photosynthate supply does not meet the demand. The model of Dewar et al. (1998) is useful in describing why the plant communities in this study achieved their new steady-state values of CUE after a few days and provides a conceptual understanding of why CUE changed when PPF was suddenly changed from previous steady-state levels. The rate at which the plants return to steady state conditions depends upon the species.

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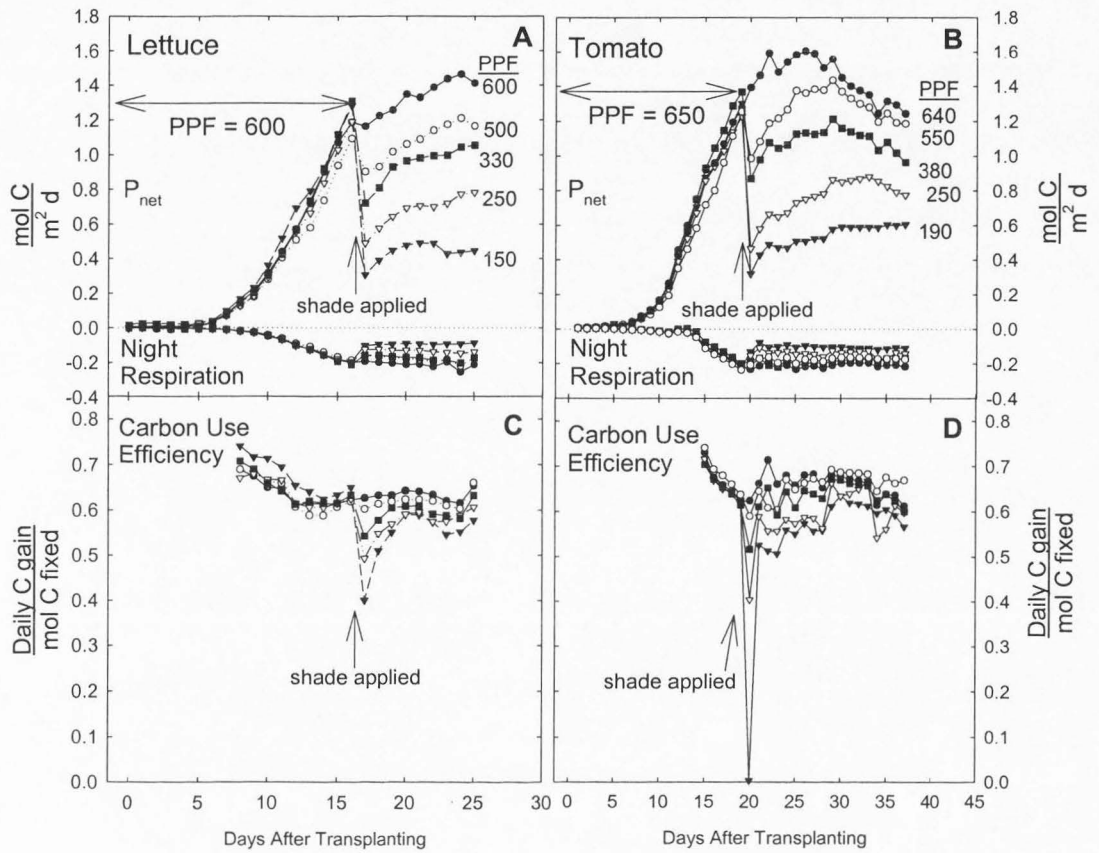
Figure 4-1. Net photosynthesis and night-time respiration of lettuce canopies (A) and tomato canopies (B). The PPF corresponds to the light in which canopies were grown after shade was applied. Carbon use efficiency is also shown for lettuce (C) and tomato (D). Data are expressed as moles of CO_2 per square meter of ground area per day.
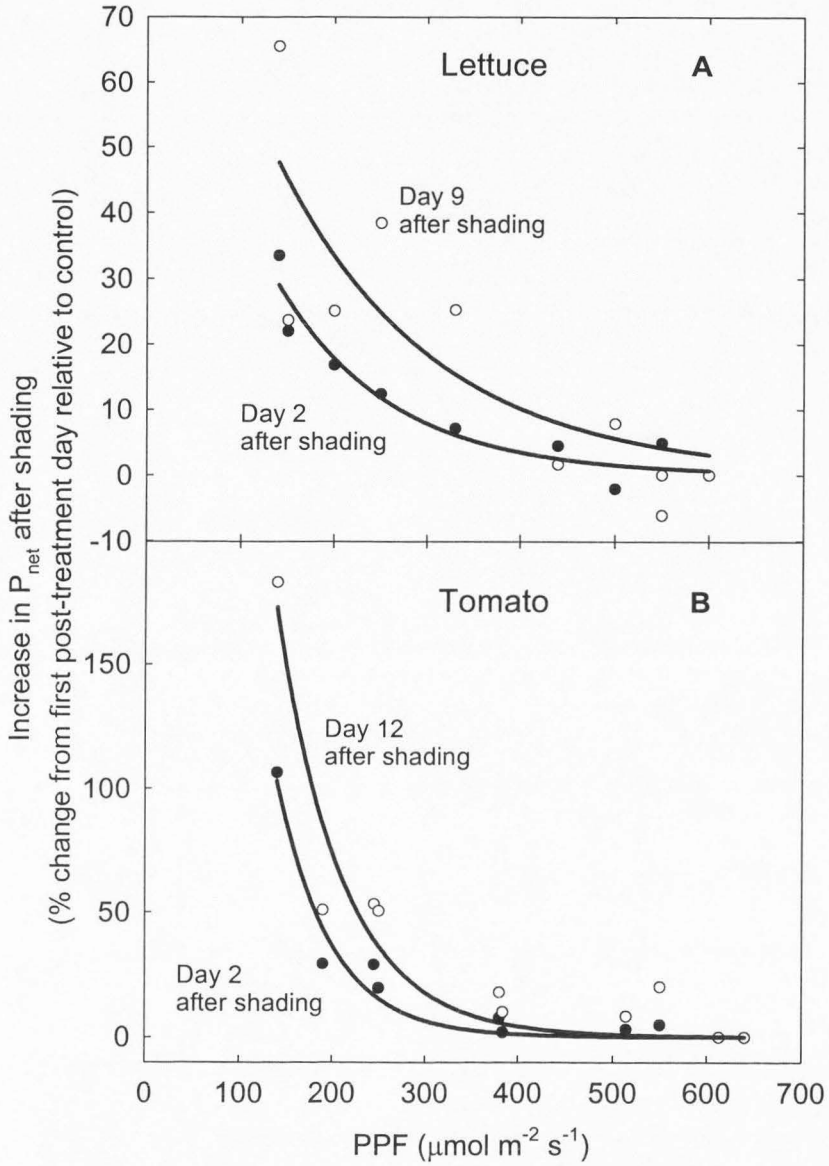
Figure 4-2. Recovery of net photosynthesis (P_{net}) recovery for lettuce and tomato. These data are from all ten chambers, and are expressed as a percent of initial P_{net} after shading treatments were imposed.
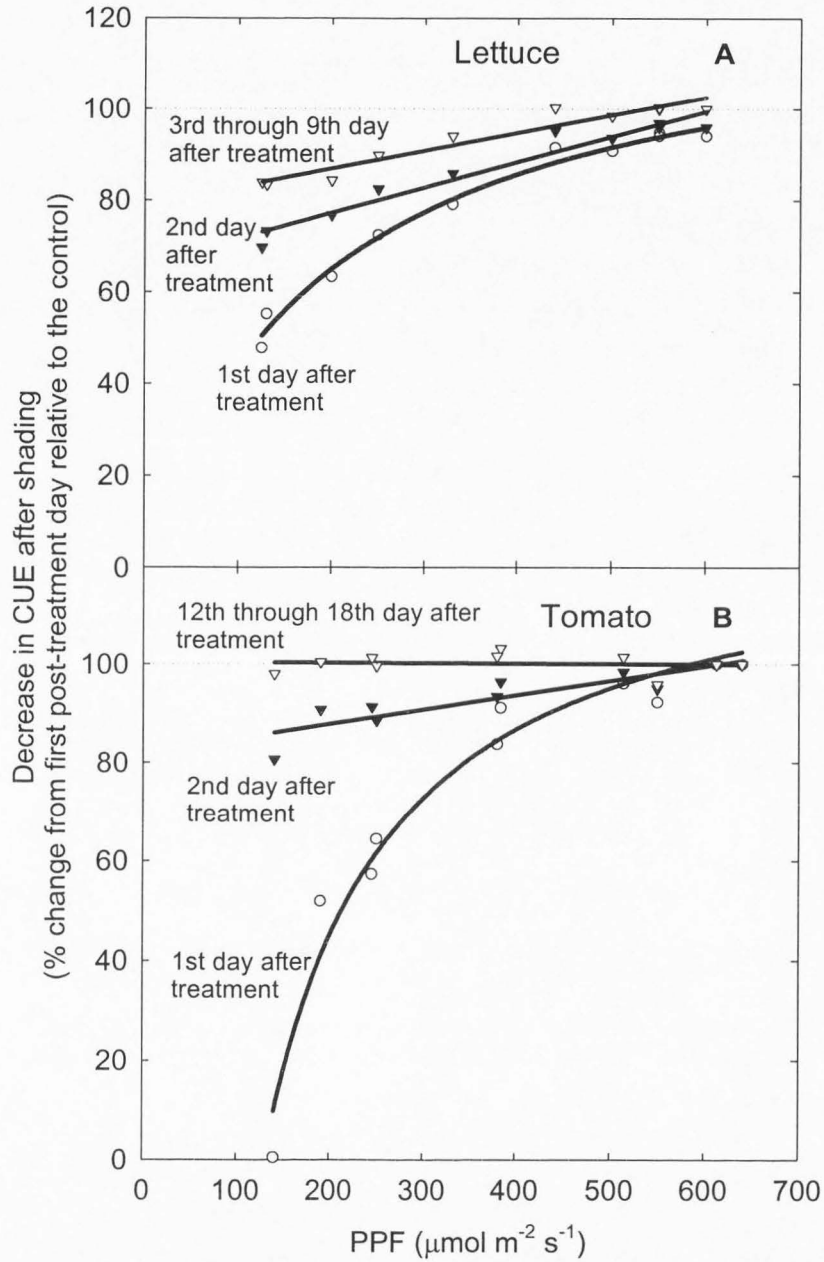
Figure 4-3. Carbon use efficiency of lettuce (A) and tomato (B) expressed as a percent of initial value and normalized as a percent of control.
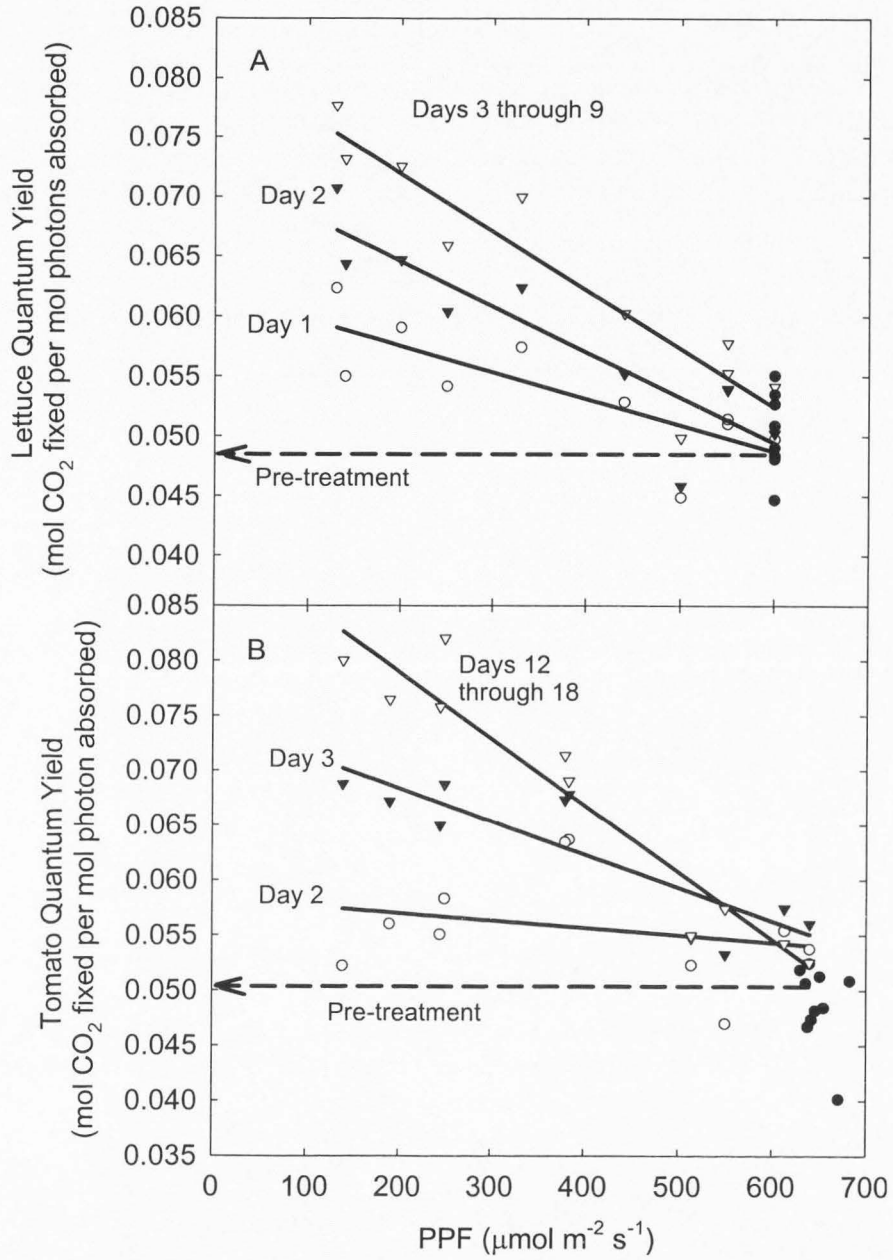
Figure 4-4. Quantum yield of lettuce (A) and tomato (B) canopies before and after shade application.
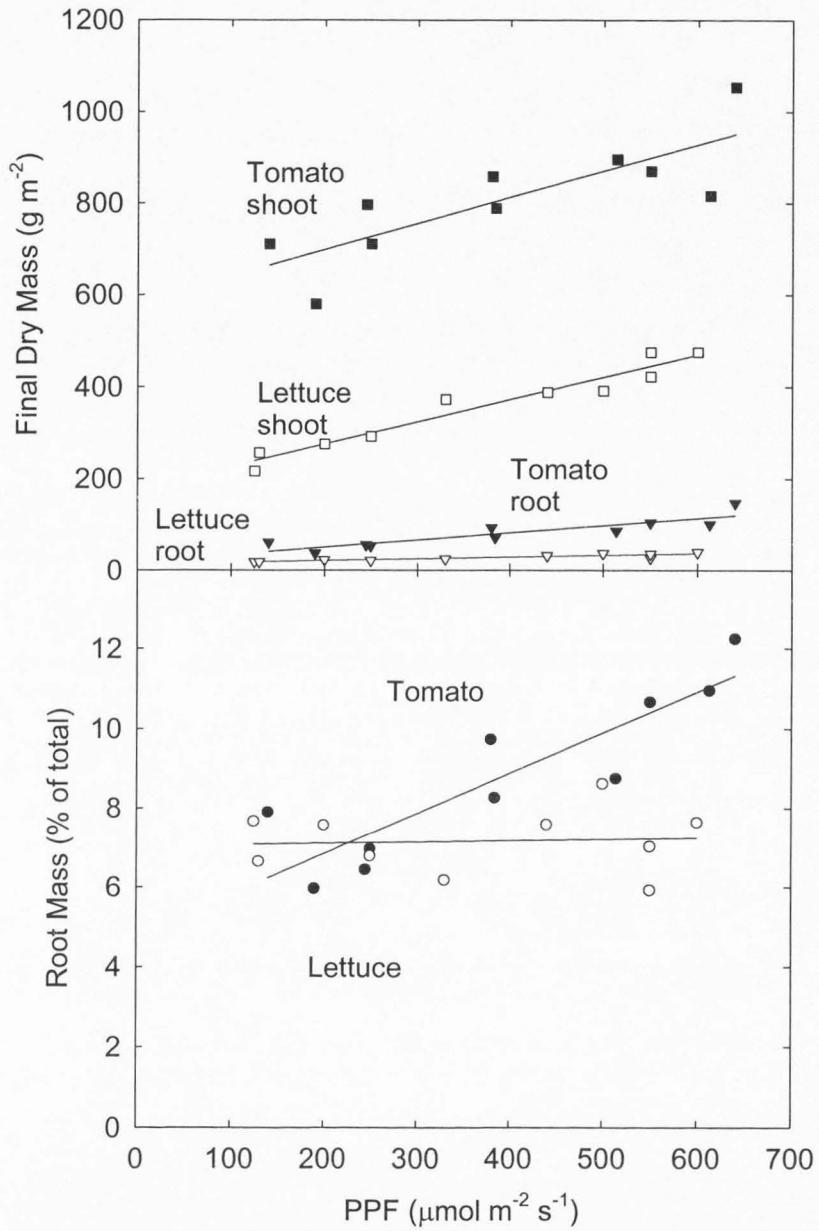
Figure 4-5. Final dry mass (A) and percent root mass (B) for lettuce and tomato.

CHAPTER 5

ACCLIMATION OF TOMATO TO HIGH AND LOW

TEMPERATURE AND LIGHT[4]

Abstract

Plants experience fluctuating light and temperature, and species acclimation defines

both their ecological niches and optimal growth ranges. Interactions between light and

temperature may be evident because of covariance of photosynthesis and respiration with

these parameters. It is, therefore, important to determine the effect of a change in light

and temperature on respiration and carbon use efficiency both separately and together.

Separating respiration into growth and maintenance components and tissue analysis

revealed that carbon use efficiency has the capacity to acclimate to changes in light

through a reduction in the maintenance coefficient, an increase in the growth coefficient,

and relatively less partitioning of N in protein. Temperature had no significant effect in

determining either maintenance or growth coefficients. These data suggest that carbon

use efficiency can be maintained at a high level provided

carbohydrate supply can meet respiratory demand, even in low light.

Introduction

Plants experience fluctuating light and temperature during the course of a single day

and across the growing season. How species acclimate to such fluctuations through time

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[4]Coauthored by Bruce Bugbee.

helps define both their ecological niches and optimal growth ranges. Photosynthesis and respiration, which define biomass gain, are influenced by light and temperature. Temperature effects photosynthetic efficiency or quantum yield, and light affects both rate and efficiency of photosynthesis. Respiration has long been reported to increase exponentially with temperature, while light either decreases respiration through photoinhibition (Atkin et al., 2000; Sharp et al., 1984) or stimulates respiration through increased carbohydrate supply (Azcón-Bieto and Osmond, 1983; Moser et al., 1982).

Photosynthesis is much better understood than plant respiration. Respiration is classically divided into growth and maintenance fractions (McCree, 1974), with different processes categorized under either growth or maintenance depending on particular definitions used by authors (see Amthor, 2000 for complete discussion of different assumptions of growth and maintenance respiration). The divisions between growth and maintenance processes are somewhat arbitrary given that there is no biochemical distinction between ATP pools for growth or maintenance. In this study, we use the growth-and-maintenance-respiration paradigm as defined by Amthor (2000). Growth is, therefore, assumed to be dependent on the type of biomass being synthesized and growth rate, while maintenance respiration is influenced by temperature and plant size. Most values in the literature for maintenance respiration are usually below 5% of existing biomass per day (Lavigne and Ryan, 1997; Ryan et al., 1995; Adu-Bredu et al., 1996), while growth respiration is in the range of 0.13 to 0.43 mol CO_2 respired per mol carbon in the new biomass, depending on plant composition (Amthor, 2000).

Carbon use efficiency (CUE) is a calculated term that describes the relationship

between photosynthesis and respiration, and can be thought of as the efficiency of mass accumulation in plants. It is a ratio of the mass gained after a full 24-h period (net photosynthesis minus night respiration, or daily carbon gained, DCG) to carbon fixed during the day (gross photosynthesis, P_{gross}). Because it is a ratio, it is less sensitive to a change in either DCG or P_{gross}; however, it provides insight concerning how effective the plant is at preserving the carbon fixed during the day.

Although detailed models describing environmental influence on photosynthesis and respiration are available, the models typically predict that CUE is insensitive to changes in light or temperature, both of which influence photosynthesis and respiration in different ways. In fact, limited variation in CUE is recommended as an 'unforced outcome of mechanistic models' (Cannell and Thronley, 2000). In the model of Dewar et al. (1998) that describes carbon and light use efficiencies at the whole leaf and plant level, constant CUE was the result of stored reserves and available substrate for growth and respiration being balanced across several days. The pool of carbohydrate available for growth and respiration is typically large enough to negate small temporary changes in the environment, and the result is constant CUE. The model was tested only for variable light conditions. Variations in temperature were not modeled in that study, but were tested previously (Chapter 2). In those studies, CUE increased when night temperature decreased. The resulting change in CUE was caused by a one-time temperature change for the duration of the study, which resulted in permanent changes in carbohydrate pools available for growth and respiration. This resulted in a new CUE value.

In Chapter 4, lower light decreased CUE by as much as 80% of pretreatment values.

Values of CUE eventually returned to at or near pretreatment levels, depending on the species tested. Using the model of Dewar et al. (1998) to explain the results, carbohydrate pools presumably adapted to new steady-state levels, thereby allowing CUE to return to steady levels. Interestingly, lettuce did not return to pretreatment levels, but to a new lower steady value. The model of Dewar et al. (1998) was constructed assuming non-limiting conditions, and if those conditions are not met, then new carbohydrate supply (i.e., lower light) does not meet the demand (i.e., existing biomass) and CUE may adapt lower. It is not known how altering *both* light and temperature will influence CUE.

The purpose of this study was to investigate the influence of light and temperature on the growth of tomato (*Lycopersicum esculentum* Mill.). I wanted to evaluate why there may be no change in CUE, or if there is a change, how and why it changes. Furthermore, interactions between light and temperature may be evident because of the co-variance of photosynthesis and respiration with these parameters. Because I also wanted to examine the growth, stress, and subsequent recovery from a more mechanistic basis, I separated respiration into growth and maintenance components. In doing so, insight hopefully will be gained into existing models used for predicting crop yield and the underlying processes driving those models.

Materials and Methods

Experimental Design

Seeds of tomato ('Micro-Tina') were germinated and transplanted after five days into gas-exchange system chambers at a density of 106 plants m^{-2}. High densities were used to obtain rapid canopy closure. Additionally, the dwarf tomato cultivar 'Micro-

Tina' was used so that full-sized plants would fit inside the gas-exchange chambers.

Shade treatments were arranged as a complete block design and analyzed using two-way

ANOVA (light and temperature) and linear regression. Both blocks contained a single

control chamber (photosynthetic photon flux was 300 μmol m^{-2} s^{-1} for the duration of the

trial), two with low PPF (80 μmol m^{-2} s^{-1}) or high PPF (about 600 μmol m^{-2} s^{-1}). Slopes

of lines were compared to see if their slopes were equal using the test statistic ((slope a -

slope b) - 0)/(variance of slope a) = t $_{(degrees of freedom of slope a)}$ (Neter et al., 1996).

Plant Growth Environment

A 10-chamber open gas-exchange system was used that was described previously by

van Iersel and Bugbee (2000). Each chamber is 0.5 x 0.4 x 0.9 m (L x W x H) and fully

enclosed a hydroponic tub. After transplanting, five chambers contained plants grown at

20C day/night temperature, and five chambers were grown at 30C day/night temperature.

Chamber temperature was controlled to within ±0.2C of set point, and CO_2 was

controlled to within ±2% of 1,200 μmol mol^{-1}. The concentration of CO_2 was elevated to

ensure that photosynthesis would be limited by light rather than CO_2. Root-zone

temperature was maintained by activating flexible heat-stripping wrapped around the

outside of the hydroponic tubs when the temperature fell below the set point. Hydroponic

solution was bubbled with the same CO_2-enriched air as that used in the canopy. The

CO_2 exchange of each of 10 different whole canopies was monitored once every 10

minutes.

Manual adjustment of pH on a daily basis resulted in a 1 pH unit day-to-day range.

The pKa of carbonate is 6.2, which means that 50% of the carbon dissolved in the water

is in the carbonate form and 50% is CO_2. Due to the limitations of our pH control method, a 1 pH unit range during the day has the potential to cause significant fluxes in and out of the nutrient solution. For this reason, the pH of the hydroponic solution was maintained between 4 and 5, which forces between 90 and 99% of the CO_2 out of solution (Monje and Bugbee, 1998). Relative humidity was maintained between 60 and 85% for the duration of the trials. The photosynthetic photon flux (PPF) was provided by water-filtered HPS lamps that initially provided 300 μmol m^{-2} s^{-1} ($\pm$5%) for all chambers until canopy closure. Both trials were run using a 16-h photoperiod to initially provide 17.28 mol photons per m^2 per day.

Shade was applied two days after canopies closed, which occurred 16 days after transplanting for the canopies at 30C and 24 days after transplanting for the canopies at 20C. Shade was applied using a neutral-density window screening (10-mesh) positioned on the top of the chambers that reduced PPF by 50% with each complete layer of window screening and an opaque black plastic draped over the tops of the chambers for low light treatments. The PPF was measured twice weekly with a line-quantum sensor (Model LQSV-ELEC, Apogee Instruments, Inc., Logan, UT) that averaged PPF across the top of the canopy. Shade cloth was adjusted at those times to maintain shade to original shade level, and shading continued for 20 days after shade application. Side lighting was reduced by wrapping each chamber in a reflective curtain and adjusted so that the top of the curtain was level with the top of the canopy.

Calculations

The ratio of carbon gained per day to total carbon fixed is CUE, which is defined as:

$$CUE = DCG / P_{gross}$$

where DCG is daily carbon gain and P_{gross} is gross photosynthesis. Using the measured CO_2 exchange rates of P_{net} (net photosynthesis, mol C m^{-2} d^{-1}) and R_n (night-time respiration, mol C m^{-2} night^{-1}; night is the dark period following the photosynthesis period, which together make up one 24-h period), DCG can be calculated as:

$$DCG = P_{net} - R_n$$

where P_{net} is net photosynthesis and R_n is night respiration. Gross photosynthesis (P_{gross}) is a calculated term that incorporates both the net C fixed (P_{net}) and the C that is simultaneously being respired. Because day-time respiration (R_d) can not be measured directly, P_{gross} is calculated as the sum of P_{net} and some percentage of night-time respiration rate. Different studies have indicated that R_d can remain high during the day due to higher carbohydrate content during the day (Azcón-Bieto and Osmond, 1983), or can be lower due to some type of light-inhibition of respiration (Atkin et al., 2000; Sharp et al., 1984). I assumed that R_d is occurring at the same rate as R_n, as is often assumed for whole plants, so that R_d would be defined as:

$$R_n * (\text{time in light})/(\text{time in darkness}).$$

Thus, for a 16-h photoperiod, $R_d = R_n * 2$. In these equations, respiration is designated as a positive value (i.e., mass respired). Therefore, P_{gross} can be calculated as:

$$P_{gross} = P_{net} + R_d.$$

Sensitivity analysis of CUE to changes in R_d indicates that R_d can change by as much as 50% higher or lower than R_n with a resulting change in CUE of only 0.08 or 12% (assuming a typical CUE of 0.65). Therefore, the assumption of constant day and night

respiration rate has little impact on the calculated value of CUE. Changes in CUE relative to the control are more important than absolute changes to CUE with shade application.

Quantum yield (QY) was calculated for each canopy after canopy closure. The PPF incident upon the canopies was measured and 95% of that was assumed to be absorbed. The P_{gross} for the day period (mol C m^{-2} d^{-1}) was then divided by total photons absorbed (mol photons m^{-2} d^{-1}) to give QY (mol C fixed per mol photons absorbed).

After treatments were imposed, data were expressed as a percent of their initial value, then normalized to the control in the following manner:

$$(\text{Posttreatment}_a \; \text{day}_b \; / \; \text{Pretreatment}_a \; \text{value}) \div$$

$$(\text{Posttreatment}_{control} \; \text{day}_b \; / \; \text{pretreatment value}_{control})$$

where posttreatment$_a$ indicates the posttreatment value of parameter a (i.e., CUE, P_{net}, and R_n), day$_b$ indicates the day after treatment, pretreatment$_a$ value is the value of the parameter of interest on the day before treatments began, posttreatment$_{control}$ day$_b$ is the posttreatment value of the parameter of interest on the same posttreatment day, and pretreatment value$_{control}$ is the pretreatment value of the parameter of interest the day before treatments began. By doing this transformation, treatment effects on a given canopy are accounted for in the numerator, and the effects relative to the control and ontogeny can be

assessed in the denominator.

Plant Tissue Analysis

Upon harvesting, tomato plants were separated into leaves, stems, roots, and fruit (if

present). Tissue was weighed and dried in an forced-air oven at 80C for 72 hours. Dry

biomass was subsequently weighed, ground, and subsampled for analysis. Samples

weighing 0.2 g were analyzed for percent C, H, and N with a LECO analyzer (Utah State

Plant and Soils Analysis Laboratory), and samples of 1.0 g were used for analysis of other

nutrients (ICP analysis, Plant and Soils Analysis Laboratory, Utah State University).

Nitrate was analyzed with a 0.2 g sample placed in a 50-ml solution of 0.05 M $Al_2(SO_4)_3$.

The tissue and solution were shaken four times during the 1-h extraction period. The

solution was measured with a NO_3^- selective and an associated reference electrode (Model

930700 and Model 900200 Thermo Orion, Beverly, MA). The readings were then

converted from volts to NO_3^--N from a previous calibration curve.

Growth and Maintenance Estimates

The model of Hesketh et al. (1971) was used to separate respiration into growth and

maintenance components. This model is based on the classic respiration model made

popular by McCree (1974) where respiration is a linear function of biomass amount and

new growth:

$$R = cW + mG$$

where R is total respiration, c is some fraction of existing biomass (W) devoted to

maintaining existing biomass, and m is some fraction of new biomass (G) devoted to

growth respiration. If both sides are divided by W:

$$R/W = cW/W + mG/W$$

the equation simplifies to:

$$specific\ respiration = c + m*RGR$$

or specific respiration, which is a linear function of relative growth rate (RGR) times the growth coefficient plus the maintenance coefficient. Using CO_2 continuous gas exchange measurements from whole plants permitted an estimation of specific respiration and RGR during plant canopy growth.

Results

Large differences in the rate of canopy closure occurred between the two temperature treatments. As a result, the number of days required for canopy closure differed between temperatures. The warmer treatment reached canopy closure on Day 16, while the cooler treatment reached canopy closure eight days later. The different temperatures also caused different development rates; the warm treatment required only about 25 days for canopies to initiate flowering, and the cool canopies required about 35 days for the first flower to appear.

30C Treatment

Photosynthetic rate decreased from 0.74 mol C fixed m^{-2} d^{-1} to 0.12 mol C fixed m^{-2} d^{-1} (84% reduction) the initial day after reducing PPF from 300 to 80 μmol m^{-2} s^{-1} (73% shade) (Figure 5-1A). Photosynthesis increased from 0.76 to 1.47 mol C m^{-2} d^{-1} (93% increase) the initial day after increasing PPF from 300 to 600 μmol m^{-2} s^{-1} (100% increase). On subsequent days, photosynthetic rate slightly increased relative to initial pretreatment values and relative to the control in the shaded canopies ($t = 2.6$, $df = 8$, $P < 0.025$ from Day 1 to Day 2; $t = 3.77$, $df = 8$, $P < 0.005$ from Day 2 to Day 12), and slightly decreased relative to initial pretreatment values and control in the high light

environment (Figure 5-2A).

Respiration rate decreased from -0.11 mol C respired m^{-2} $night^{-1}$ to -0.055 mol C respired m^{-2} $night^{-1}$ (50% reduction) the initial day after reducing PPF from 300 to 80 μmol m^{-2} s^{-1} (73% shade) (Figure 5-1A). Respiration increased from -0.11 mol C respired m^{-2} $night^{-1}$ to -0.17 mol C respired m^{-2} $night^{-1}$ (52% increase) the initial day after increasing PPF from 300 to 600 μmol m^{-2} s^{-1} (100% increase). On subsequent days, respiration rate continued to decrease relative to initial pretreatment values and relative to the control in the shaded canopies (t = 2.411, df = 8, P < 0.025 from Day 1 to Day 2, not significantly different after Day 2), and increased slightly in the canopies receiving high light relative to initial pretreatment and control values (Figure 5-2B).

Carbon use efficiency decreased to 0.31 from 0.65 after the first day of shading (Figure 5-1C, 5-2C, 5-3B). Carbon use efficiency recovered completely after about 10 days, with most recovery occurring after the first three days of shade (Figure 5-1C and 5-2C). In the canopies receiving high PPF, the CUE initially increased from 0.65 to 0.72 on the first day after treatment. Subsequent days showed an exponential decline in CUE with a return to the same values as before treatment after about 10 days (Figure 5-3A).

20C Treatments

Photosynthetic rate decreased from 0.72 mol C fixed m^{-2} d^{-1} to 0.12 mol C fixed m^{-2} d^{-1} (or 83%) the initial day after reducing the PPF from 300 to 80 μmol m^{-2} s^{-1} (73% shade) (Figure 5-1B). Photosynthesis increased from 0.85 mol C fixed m^{-2} d^{-1} to 1.39 mol C fixed m^{-2} d^{-1} (or 63%) the initial day after increasing PPF from 300 to 600 μmol m^{-2} s^{-1} (100% increase). On subsequent days, photosynthetic rate slightly increased relative to

initial pretreatment values and relative to the control in the shaded canopies, and slightly

decreased relative to initial pretreatment values and control in the high light treatment

(Figure 5-2A).

Respiration rate decreased from -0.12 to -0.071 (or 41%) the initial day after

reducing the PPF from 300 to 80 μmol m^{-2} s^{-1} (73% shade) (Figure 5-1D). Respiration

increased from -0.14 to -0.18 (or 32%) the initial day after increasing PPF from 300 to

600 μmol m^{-2} s^{-1} (100% increase). On subsequent days, respiration rate continued to

decrease relative to initial pretreatment values and relative to the control in the shaded

canopies, and continued to increase slightly relative to initial pretreatment and control

values in the canopies receiving high light (Figure 5-2B).

After the first day of shading, CUE decreased from 0.64 to 0.18 (Figure 5-1D, 5-2C,

and 5-3B), and recovered completely after about 10 days, with most recovery occurring

after the first three days after shading. This pattern matched that of the 30C treatment.

In canopies receiving high PPF, the pattern was different from that of the 30C

treatment (Figure 5-3A). Carbon use efficiency initially increased from 0.64 to 0.68 on

the first day after treatment. A best fit regression for subsequent days indicated a linear

decrease, which was significant, but relatively low correlation (P < 0.001; r^2 = 0.68). Our

analysis does not eliminate the possibility of an exponential decrease in CUE with a low

slope. Both the warm and cool treatments were shaded or had high light for 20 days, so it

is not known if the pattern is exponential or linear beyond 20 days.

Growth and Maintenance Estimates

Relative growth rate and specific respiration were highly correlated within each

chamber ($F = 226.1$, $df = 23$, $P < 0.001$, r^2 of at least 0.91) (Figure 5-4A and 5-4B). Graphs are separated according to chamber temperatures to facilitate comparisons between PPF levels. Shade treatments had low values of RGR, so the distance the regression line must be extrapolated in order to estimate the intercept is a relatively smaller distance than those treatments with higher RGRs. Nevertheless, correlations for these treatments were high.

Estimates of the growth coefficient (slope) increased with decreasing PPF, while the maintenance coefficient (intercept) decreased with lower PPF (Table 5-1). ANOVA indicated a significant relationship between both the estimate of the growth coefficient and the maintenance coefficient with PPF. Comparison of group means by Tukey's test indicated that the low (80 μmol m^{-2} s^{-1}) and high (600 μmol m^{-2} s^{-1}) PPF treatments differed significantly from one another in both growth and maintenance estimates, but other group means did not differ significantly. Temperature did not affect the growth ($P = 0.91$) or maintenance coefficient ($P = 0.51$).

Tissue Analysis

Both temperature and PPF significantly affected leaf C content ($P < 0.001$) (Table 5-2 and 5-3). Higher light and warmer temperatures led to higher percent C. A significant interaction was observed between PPF and temperature on leaf carbon content ($P = 0.003$). Stem and root C content also increased significantly with high PPF (Table 5-2).

The N content in the leaf decreased with higher PPF, but was not directly related to temperature (Table 5-2 and 5-3). There was a significant light and temperature

interaction, however, which indicated that N content was different under high and low light depending on temperature. The N content in the stem was not influenced by either light or temperature. Cooler temperature decreased root N content, possibly due to the influence of temperature on uptake rates. Nitrate content of the leaf and stem was only influenced by PPF, with lower PPF resulting in much higher NO_3^- content, while low NO_3^- content was observed at high PPF. Stem NO_3^- content also decreased at high PPF.

Quantum Yield

There was a significant difference in QY between PPF levels with the highest QY occurring under the lowest light level, and the lowest QY occurring under the highest PPF (Table 5-4). There was marginal significance in QY in response to temperature (P=0.08), with the 30C treatment having a slightly higher QY than the 20C treatment.

Discussion

Models that separate respiration into growth and maintenance components assume no effect of temperature on the growth coefficient but a strong effect on the maintenance coefficient (Heuvelink, 1995). This effect is usually described with a Q_{10} term of about 2.0 (Witowski, 1997; McCullogh and Hunt, 1993; Edwards and Hanson, 1996). If these models are accurate, we should have seen a doubling of maintenance respiration between the two temperature treatments; however, no effect of temperature on the maintenance respiration coefficient was detected in this study. This differs from the results of other studies that reported no effect of temperature on maintenance respiration, but nevertheless assume a Q_{10} of 2 for the maintenance coefficient (Heuvelink, 1999)

Each canopy developed in a constant temperature environment with day, night, and root zone temperatures all being identical. These conditions do not simulate a typical plant growth environment and, therefore, may not be a true reflection of how plants respond in a normal, fluctuating environment. The same can be said for short-term studies on plant parts. Taken together, the two methods provide different information about plant response to temperature. Our results indicate that maintenance respiration does not respond to temperature. However, these plants were never stressed by fluctuating temperatures and, therefore, had no requirement to increase or decrease their maintenance in response to the environment. That is, maintenance respiration may have acclimated to the growth environment and estimating a Q_{10} from different plants grown in different environments may not be a true representation of how maintenance respiration responds to a fluctuating environment. Conversely, a plant that undergoes fluctuations on a daily or hourly basis likely has acclimated to such fluctuations and, as a result, has less maintenance respiration than a one-time change in temperature in the leaf, root, or fruit with an apparent Q_{10} of 2 or higher. Models may need to reflect the dynamic nature of a temperature response (i.e., Q_{10} value) by scaling it to how often and severe a temperature fluctuation a plant or canopy experiences through time (seconds, minutes, hours, or days).

On the other hand, PPF changed drastically a single time during the trial. The ANOVA indicated that different PPF resulted in changed maintenance and growth respiration coefficients. The maintenance coefficient roughly doubled with each increase in PPF, but a pairwise Tukey's test indicated that only the low PPF and the high PPF were different. All estimates were on the low end of the range of values typically

reported for maintenance requirement (Amthor, 2000). Our trial was run in elevated CO_2, which reportedly causes reductions in maintenance requirements, due in part to decreased Rubisco concentrations of plants grown in elevated CO_2 (Bunce, 1995).

The growth coefficient decreased with increasing PPF, suggesting that plants under high PPF synthesized simpler compounds such as sugars and starches, whereas plants in the low PPF treatments were synthesizing more complex compounds (Amthor, 1989). Plants grown under low PPF tend to synthesize more light-harvesting compounds, less storage compounds, and have less Rubisco (Zhao and Oosterhuis, 1998), whereas plants grown under high PPF tended to accumulate starch in leaves and have more Rubisco (Goldschmidt and Huber, 1992; Yelle et al., 1989). These growth coefficients are consistent with those physiological changes as plants acclimate to low and high PPF.

Carbon use efficiency acclimated to low PPF in a similar manner as previously reported (Chapter 4). Some of the acclimation could have been the result of differences in QY after shade was imposed. The low light plants had greater QY, while the high PPF treatments were less efficient photosynthetically. The large decrease in CUE on the initial day after shading indicated that plants were also less efficient at conserving newly fixed carbon immediately after shading. The CUE did not decrease as much as previously reported. In that study, however, plants were shaded the same percentage (~80%), but were changed from 600 μmol m^{-2} s^{-1} down to 125 μmol m^{-2} s^{-1}, a larger absolute change in PPF. The following day, CUE greatly increased, back to within about 80% of its initial, pretreatment value. I attribute this large initial change in CUE to a temporary imbalance of stored C that permits respiration to continue at a high rate.

However, as those reserves are consumed, respiration decreases in balance with photosynthesis. No interaction was observed between temperature and PPF, which suggests that these stored reserves were consumed at roughly equal rates or were mobilized equally well at different temperatures when shaded. This explanation fits the model of Dewar et al. (1998) for steady CUE values under non-limiting conditions. Provided the growth and maintenance requirements are both met, CUE should be the same in different constant environments.

In estimating growth and maintenance coefficients, I assumed that growth and maintenance were unchanged during this study. I, therefore, assumed that a Michaelis-Menton type of relationship existed between CUE and RGR; that is, a change in RGR resulted in a change in CUE. While this may be the case, RGR apparently remains high enough to not substantially affect CUE and indeed remain near its maximum value (value under optimal conditions). This would happen if the maintenance requirement is extremely low, as predicted for this trial. It also provides further evidence that if the demand for substrate for growth and maintenance is met with the substrate supply (even in very low RGRs), CUE can remain high.

The response of CUE to a change from low to high PPF was different for the two temperature environments used in this study. Both showed an initial increase in CUE the first day after PPF increase, and the warmer treatment declined back to initial pretreatment levels after a few days. This suggests differences in starch mobilization at different temperatures, but that switching from high to low PPF had no such effect at the different temperatures. Starch synthesis in cereal grains is known to be temperature

sensitive, and warm temperature in wheat (*Triticum aestivum*) during the grain filling stage reduced seed mass because of lower rates of starch accumulation (Bhullar and Jenner, 1986) and decreased duration of grain fill (Tashiro and Wardlaw, 1988). Differences in CUE may be the result of a prolonged imbalance of supply and demand due to starch synthesis in leaves.

Several attempts have been made to correlate maintenance respiration requirement with N or protein content (Ryan et al., 1996; Dewar, 1996). It is believed that N status is a reflection of protein amount and, therefore, an indirect measure of protein amount that needs to be maintained. As a result, the higher the N content, the higher the maintenance cost. In our study, leaf N content decreased with increased light, which is opposite of what would be expected if N content were related to maintenance respiration. Even after subtracting the NO_3^- fraction from total N, (a number that should reflect both protein N and amino acid N), the remaining N forms still decrease with increasing PPF. The stem had a weaker response of N to PPF, while root N remained virtually unchanged. A higher fraction of NO_3^- N may indicate a smaller requirement of maintenance respiration, possibly maintaining ion gradients rather than functional proteins.

Surprisingly little difference in N or NO_3^- content was observed between the two temperature environments. Kafkafi (1990) reported about a 33% increase in leaf NO_3^- concentration as temperature increased from 24 to 34C and about a 50% decrease in root NO_3^- as the temperature increased from 24 to 34C. This was attributed to less NO_3^- transport from the root to the shoot under the warmer temperatures.

The differences in carbon content in different PPF tended to reflect the possible

starch versus no-starch accumulations under high and low light. The differences in percent C measured in leaves, stems, and roots possibly reflected how much and where starch was accumulating. Slightly more C was observed in leaves grown in warm than in cool temperatures, while there was more C in roots of plants grown under cool than in warm temperatures. No differences were observed in stem C.

Together, these data indicate that both growth and maintenance respond to fluctuations in light more so than differences in temperature. Because respiration, in general, and maintenance respiration, in particular, has widely been measured to have a temperature response, we suggest that the temperature response is a function of not only the magnitude of temperature change, as is usual in models, but also how often the change is incurred and for what duration plants experience the change. Our data also indicate that CUE can change in response to the environment. However, as long as the demand for growth and maintenance is met by newly fixed carbon, CUE can remain as high as values obtained under optimal conditions. This may be caused by either exceptionally low maintenance requirements, or high RGR under somewhat limiting conditions.

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Table 5-1. Estimates of growth and maintenance coefficients for the average of the different PPF and temperature treatments. Degrees of freedom for PPF in all ANOVA is 2 and the degrees of freedom for temperature in all ANOVA is 1.

	PPF (μmol m^{-2} s^{-1})			Temperature (C)	
	80	300	600	20	30
Growth (mol C$_{resp}$ mol C$_{growth}^{-1}$)	0.587	0.506	0.379	0.492	0.489
Std. error	0.02	0.03	0.02	0.02	0.02
Significance		F = 18.7		F = 0.013	
		P = 0.009		P = 0.91	
Maintenance (mmol C mol C d^{-1})	3.39	6.47	11.6	6.56	7.77
Std. error	1.3	1.8	1.3	1.2	1.2
Significance		F = 10.85		F = 0.51	
		P = 0.024		P = 0.51	

Table 5-2. Carbon, nitrogen, and NO_3^- contents (g per kg) for the average of the different PPF treatments. Degrees of freedom for PPF in all ANOVA is 2.

	Leaf PPF (μmol m^{-2} s^{-1})			Stem PPF (μmol m^{-2} s^{-1})			Root PPF (μmol m^{-2} s^{-1})		
	80	300	600	80	300	600	80	300	600
C	351	365	389	335	356	387	353	378	390
Std. error	2.2	3.1	2.2	7.3	10.3	7.3	4.2	4.9	3.5
Significance		F = 80.1			F = 12.9			F = 23.5	
		P < 0.001			P = 0.018			P = 0.015	
Total N	63	49	40	35	33	26	43	45	42
Std. error	1.2	1.7	1.2	2.5	3.5	2.5	0.6	0.7	0.5
Significance		F = 96.0			F = 3.14			F = 6.7	
		P < 0.001			P = 0.15			P = 0.078	
NO_3^-	23	15	9	22	17	10	14	12	9
Std. error	1.2	1.7	1.2	2.3	2.6	1.9	1.3	2.4	1.3
Significance		F = 32.8			F = 10.0			F = 8.11	
		P = 0.003			P = 0.047			P = 0.062	

Table 5-3. Carbon, nitrogen, and NO_3^- contents (g per kg) for the average of the different temperature treatments. Degrees of freedom for temperature in all ANOVA is 1.

	Leaf		Stem		Root	
	20C	30C	20C	30C	20C	30C
C	353	383	358	359	380	368
Std. error	2.0	2.0	6.9	6.9	3.3	3.6
Significance	F = 108.0		F = 0.011		F = 6.579	
	P < 0.001		P = 0.923		P = 0.083	
Total N	49	52	30	32	42	45
Std. error	1.1	1.1	2.3	2.3	4.8	0.4
Significance	F = 4.35		F = 0.56		F = 18.4	
	P = 0.11		P = 0.50		P = 0.023	
NO_3^-	16	15	15	18	11	13
Std. error	1.1	1.1	2.0	1.7	1.7	1.0
Significance	F = 0.612		F = 1.29		F = 3.14	
	P = 0.48		P = 0.34		P = 0.18	

Table 5-4. Differences in quantum yield (mol C fixed per mol photons absorbed) after shade application at 20 days after treatment. Data were analyzed with a two-way ANOVA and Tukey's comparison of means within PPF level, which showed that the three PPF levels differed significantly (P < 0.005). Degrees of freedom for PPF in all ANOVA is 2, and degrees of freedom for temperature for all ANOVA is 1.

| | PPF (μmol m^{-2} s^{-1}) | | | Temperature (C) | |
	80	300	600	20	30
Quantum yield	0.0718	0.0667	0.0542	0.0631	0.0653
Std. error	0.00094	0.0013	0.00096	0.00090	0.00089
Significance		F = 89.4			F = 3.1
		P < 0.001			P = 0.080
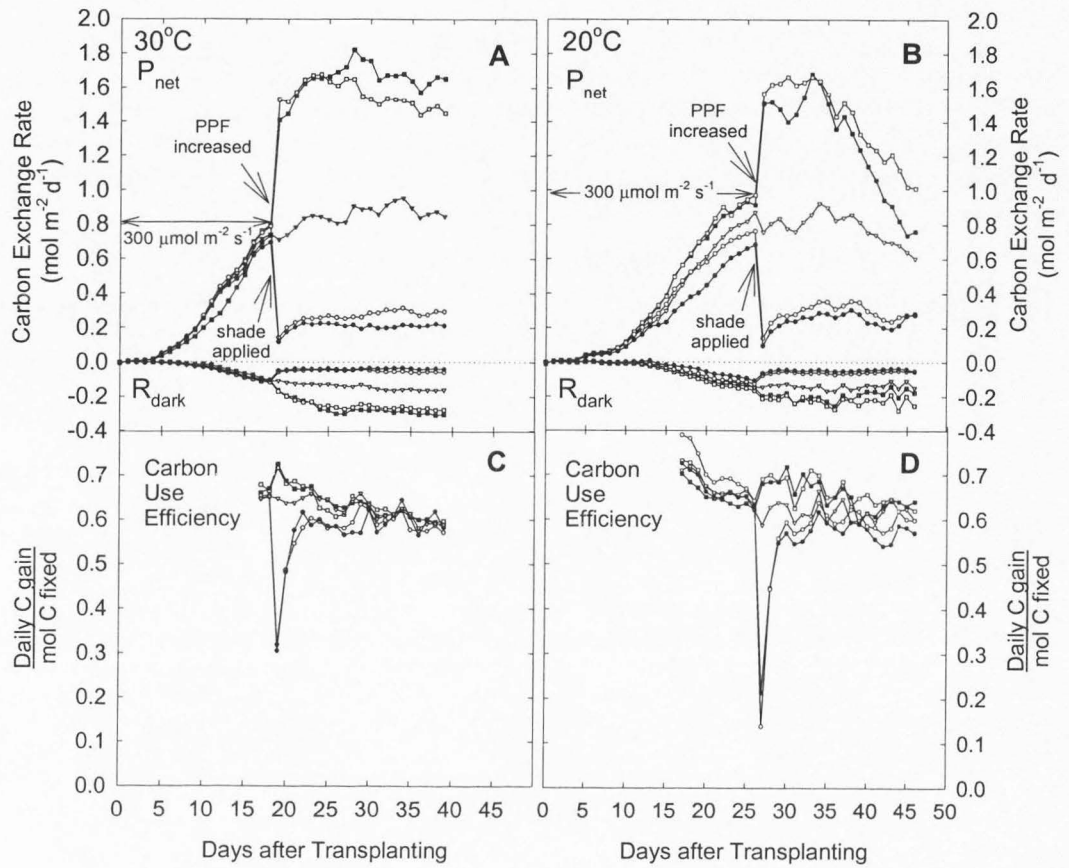
Figure 5-1. Carbon exchange rates and carbon use efficiencies for all ten chambers for both temperature treatments. PPF was changed on Day 19 for the warm canopies and on Day 27 for the cool treatment.
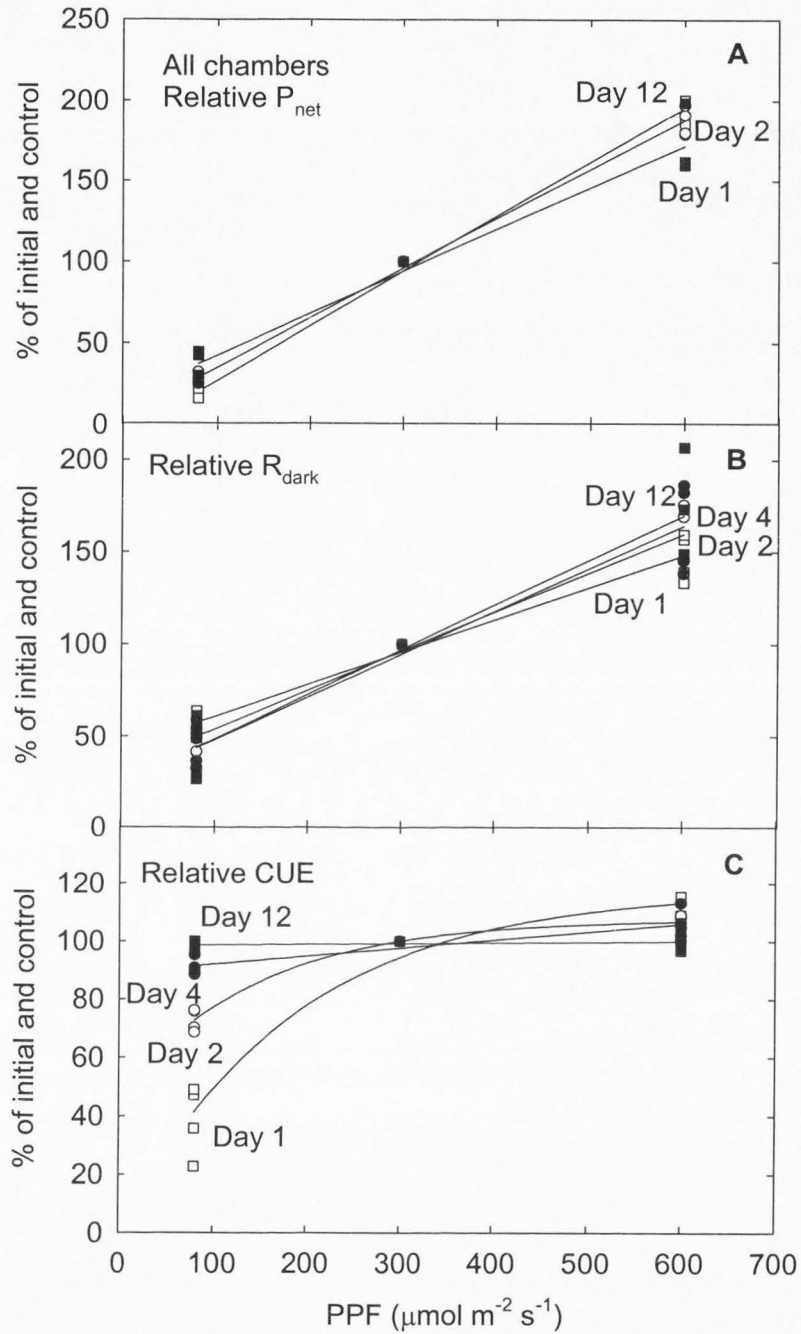
Figure 5-2. Effect of a change in PPF on net photosynthesis (P_{net}), dark respiration (R_{dark}), and carbon use efficiency (CUE). Data are shown relative to the control and pretreatment values.
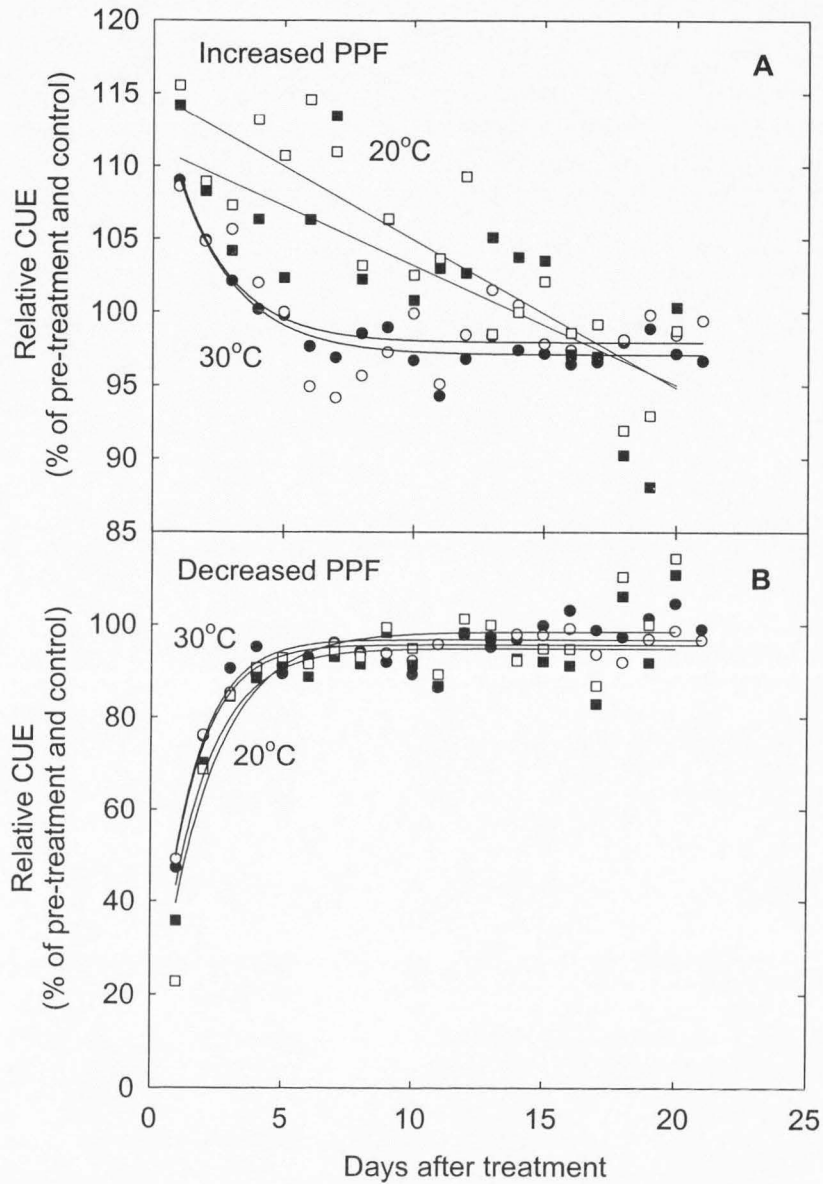
Figure 5-3. Change in carbon use efficiency (CUE) through time after increasing or decreasing the PPF. Replicate light levels at the same temperature did not differ significantly from each other.

Figure 5-4. Plots of relative growth rate (RGR) versus specific respiration in all

chambers. Correlations in each chamber were always greater than $r^2 = 0.91$ (F = 226.1, df

= 23, P < 0.0001).

CHAPTER 6

SUMMARY AND CONCLUSIONS

It is important to understand the environmental factors that influence respiration and

the physiological mechanisms that influence how plants use carbon efficiently. There is

evidence that respiratory control occurs due to demand (temperature dependency) or

supply (photosynthate availability) limitations. The relative importance of each may
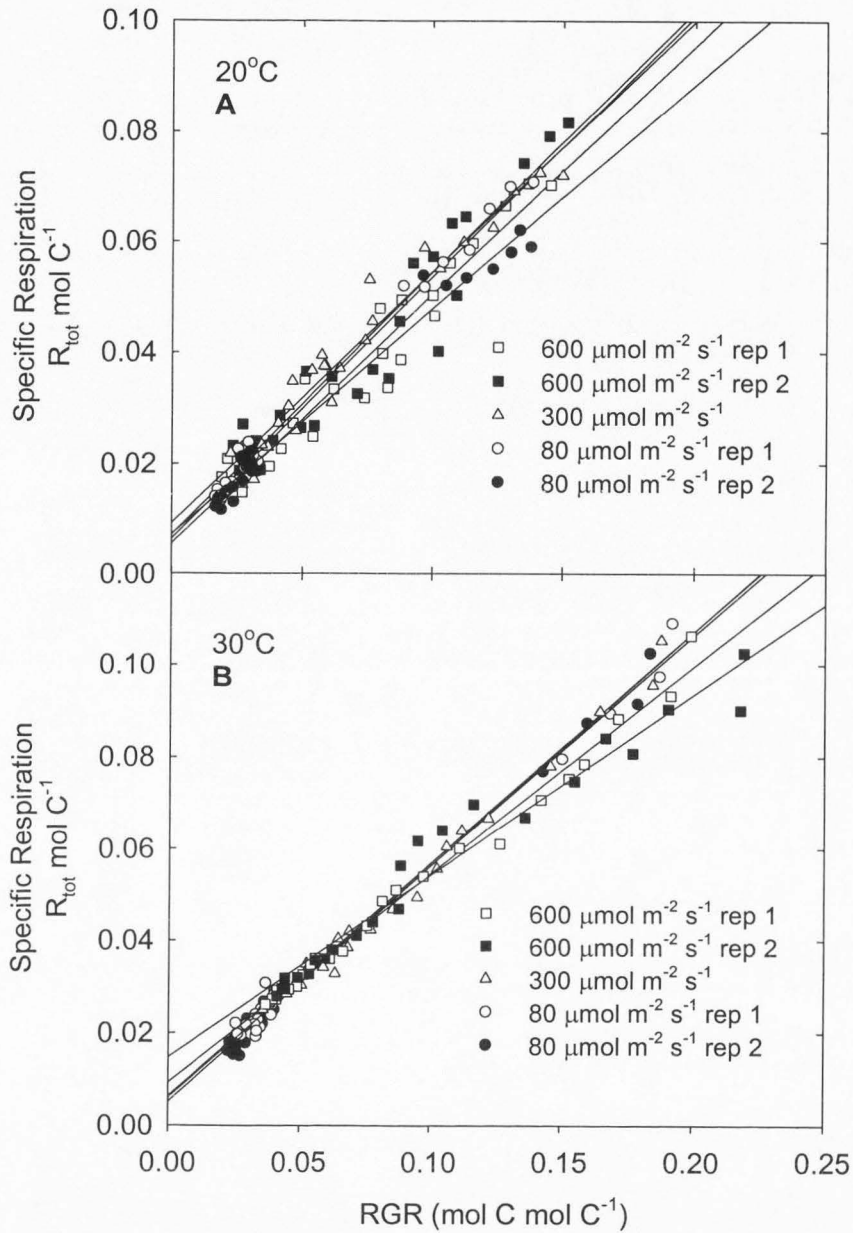
depend on a number of factors including period of time during which respiration is

measured, phase of plant development, environmental conditions, and species.

Role of Temperature

There is an abundance of literature on how temperature controls and influences

plant respiration. Unfortunately, the conclusions from this literature reached about the

role of temperature in the carbon economy of plants cannot easily be extrapolated to

longer periods of time, across species, and for whole canopies.

Initially, I had hypothesized that respiration would double for every 10C rise in

temperature, as is widely reported for respiration studies conducted using single leaves,

and that this increase in respiration would result in decreased CUE. I found, however,

that plant respiration was far less sensitive to temperature for all species tested than is

commonly reported. This is probably due to a high proportion of growth respiration,

which is not temperature sensitive, compared to maintenance respiration, which is

temperature sensitive. Furthermore, small, rapidly growing plants were used, which

should have increased the fraction of growth respiration. Breaking down respiration into

its component parts is rarely performed in analyses to explain why tissue may have higher or lower temperature sensitivity. This study should assist in interpreting the role of temperature in respiration, and provide insight into appropriate scaling of results rather than scaling from single leaf to whole plant.

Previous studies indicated that CUE remains constant regardless of temperature, or adapts when temperature is changed. However, plants grown with cooler nights than days had significantly greater CUE than the canopies receiving either constant day/night temperatures or warmer nights than compared to days when the night temperature was changed after canopy closure. The change was biologically small, however. I had hypothesized that CUE would acclimate back to pretreatment values in the days following the temperature change. The CUE did not acclimate in my study, contrary to what as was reported in the literature, but remained at the new value for the duration of the treatment, which lasted up to 20 days.

Initially I had proposed that the rate of night respiration would be correlated to the subsequent day's photosynthetic rate. I had hypothesized that elevated night-time respiration would not be efficient and would impair subsequent growth and that low night respiration would increase growth due to better respiratory efficiency. In my study, altered night respiration did not influence photosynthetic rates on subsequent days. This is in contrast to the literature that suggests substantially higher leaf photosynthetic rates after increased night temperature due to less feedback inhibition. In fact, plants with higher CUE were not larger, nor did they gain more carbon after treatments were imposed than those with lower CUE values. This is likely due to greater variability in

photosynthetic rates outweighing any potential improvement in CUE.

I wanted to further test the potential influence of temperature on CUE and respiration. Realizing that CUE was influenced when there was a difference in the day and night temperature, I hypothesized that there would be no differences in CUE of plants grown at constant day night temperature across a range of 21C to 35C. I thought that respiratory demand would be in constant proportion to the photosynthetic rate as long as the temperatures remained constant. I also hypothesized that CUE would be the same in plants that had a 5C day/night difference as other plants with the same day/night temperature difference, regardless of the average daily temperature. This was again due to respiratory demand being in constant proportion to the photosynthetic rate. I found these hypotheses to be supported by two trials performed in elevated CO_2.

If CO_2 was lowered to ambient CO_2, I hypothesized that carbohydrate supply would not meet the demand. Therefore, at low temperatures that could inhibit light capture (and therefore carbohydrate supply), or at warm temperatures with high carbohydrate demand CUE would be lower. Values of CUE were much lower at a constant day/night temperature of 35C and slightly, but significantly, lower at a constant 21C in ambient CO_2. At 35C, growth was severely stunted, and the respiratory demands may not have been met in this warm environment. At 21C in ambient CO_2, photosynthesis was efficient, as determined by canopy quantum yield, but sink strength may have been reduced, thereby, decreasing CUE. Interestingly, no such pattern was observed in the canopies in ambient CO_2 with a 5C day/night difference. This may be due to the warmest treatment (35/30C day/night) having an average daily temperature of 32C, which may

have been cool enough so that supply could meet demand. Indeed, plants grown at this temperature were not as severely stunted as those grown at constant 35C, suggesting that the temperature threshold is between 32C and 35C.

Role of Light

Studies that have exogenously applied a sugar source to excised tissue clearly showed a strong dependency of respiration on available substrate. Unfortunately, experiments at the whole plant or community level have not been performed to test this relationship. I hypothesized that on a whole plant level, the same basic patterns of reduced respiration with reduced carbohydrate supply would follow the pattern obtained with single leaf studies. The effect of low light on photosynthesis was expected to be immediate. Plants store carbohydrate, which may function as a carbohydrate reserve under stress. In low light, I proposed that photosynthesis would immediately decline, but that respiration would return to a balance with photosynthesis after consuming the carbohydrate reserves within a few days. In the tests that reduced the light level after canopy closure, photosynthesis was immediately lowered in a nearly 1:1 relationship with increasing shade. Initially after shading, the CUE was greatly reduced because respiration was high on the first night after shade application. Respiration, however, decreased during the next several days. This suggests that carbohydrate pools were large enough for respiration to continue at relatively high rates immediately after shade was applied, but eventually came back to equilibrium with the new, reduced PPF level.

Different species accumulate different forms and amounts of carbohydrate. I

hypothesized that a high starch accumulator (tomato) would require a longer period of time to consume its carbohydrate reserves than a species that had less carbohydrate and starch storage (lettuce). Values of CUE in lettuce were reduced by half after the first day of shading, whereas values of CUE in tomato were nearly zero after initial shading. This indicated that carbohydrate reserves were different in these species, and that their ability to tap into their reserves may differ. Both species reduced their respiration rates and increased photosynthetic efficiency in response to shade. Both species also reached nearly complete recovery after only a few days. However, tomato completely recovered (based on a return of CUE to pretreatment levels) after 12 days but lettuce only reached 80% of pretreatment levels at the conclusion of the trial. This suggests that respiratory demands may be different in the two species, and that some species may be better able to acclimate other physiological processes after environmental conditions change.

There have been reports of temperature influencing carbohydrate mobilization and a well established link between temperature and carbohydrate consumption. I, therefore, hypothesized that there would be an interaction between temperature and light with cooler temperatures slowing the rate of CUE acclimation to shade. However, I did not observe an interaction between shade and temperature. Both 30C and 20C canopies acclimated at the same rate with reduced PPF. Acclimation to increased PPF may have a significant interaction with plants grown in warm temperatures better able to use additional carbohydrate than plants grown in cool temperatures. This suggests possible sink limitations with plants grown in cool temperatures unable to rapidly make use of additional carbohydrate with increased light.

Growth and maintenance respiration were measured to describe how acclimation may occur in respiration with temperature or PPF changes. I hypothesized that the maintenance respiration coefficient would decrease with increasing temperature as a way to decrease respiratory demand, and that the growth respiration coefficient would decrease with lower PPF. In this study, the maintenance coefficient was not affected by temperature, and lower light not only increased the growth coefficient, but decreased the maintenance requirement as well. This response provides strong evidence that models that divide respiration into growth and maintenance components should incorporate light effects on both components, and perhaps decrease the emphasis of temperature on the maintenance coefficient.

APPENDIX

APPENDIX A

EXPLORING THE LIMITS OF CROP PRODUCTIVITY: QUANTUM YIELD,

RADIATION CAPTURE, AND CARBON USE EFFICIENCY OF LETTUCE

IN A HIGH LIGHT, TEMPERATURE, AND CO_2 ENVIRONMENT

Introduction

There have been several analyses to determine the theoretical maximum yield of a crop community (Loomis and Williams, 1963; Thornley and Johnson, 2000). For example, it was modeled and validated that wheat yield responds nearly linearly to increased photosynthetic photon flux (PPF) if all other conditions are optimized (Bugbee and Salisbury, 1988). Many crops are grown in sub-optimal conditions and maximum growth rates are sacrificed in order to improve some other characteristic of crop production (e.g. timing for the market). As a result, theoretical maximum yields have not been analyzed for several important crops and breeders have little information as to what, if anything, may be most limiting to productivity.

Most productivity studies are performed under ambient or field CO_2 concentrations. Due to an increase of photorespiration in elevated temperatures for C3 crops, quantum yield, a measure of photosynthetic efficiency and a parameter in many productivity models, is reduced in warmer temperatures in ambient CO_2. It is well established theoretically, but often ignored experimentally, that the temperature optimum for C3 species should increase several degrees under elevated CO_2 (Harley and Tenhunen, 1991; Long, 1991). Photorespiration is reduced substantially by elevating CO_2, even in warm

temperatures, and electron transport becomes the rate limiting factor in CO_2 fixation. The potential rate of electron transport continues to increase up to 30 to 35C (Farquar et al., 1980), and as a consequence, crops photosynthetic rate can be much higher in elevated rather than in ambient CO_2.

Radiation capture is also a critical component of productivity models. Leaf expansion rate is greatly influenced by temperature (Faust and Heins, 1994; Clifton-Brown and Jones, 1997) and along with leaf emergence rate, provide a plant the means to effectively capture light as it develops from seedlings. The temperature optimum for leaf expansion differs for different crops. Cotton, a warm season crop, has a temperature optimum for leaf expansion of about 31C (Reddy et al., 1993), while broccoli has a optimum of only 21C (Olesen and Grevsen, 1997). While lettuce is considered to be a cool-season crop, it is not known what its temperature optimum is for leaf expansion and radiation capture.

Carbon use efficiency (CUE) is a measure of how well a plant incorporates newly fixed carbon into new biomass. It is a ratio of weight gain to fixed carbon, or gross photosynthesis (P_{gross}). Because it integrates both respiration and photosynthetic rates, CUE may be sensitive to light, temperature, and CO_2 concentrations. Little information exists for lettuce CUE in different environments.

Lettuce is typically grown in cool temperatures (day-time temperatures between 20 to 25C) and in low light (often the maximum is 400 μmol m^{-2} s^{-1}) (Hammer et al., 1978, Swiader et al., 1992; Koontz and Prince, 1986; Jie and Kong, 1997). While adequate yields can be obtained in this environment, the main reason for these growth conditions is

to improve quality (Saure, 1998). Exceptional dry weight gain through optimization of environmental parameters means little if the crop is unpalatable. In the case of lettuce, high growth rates are sacrificed in order to obtain superior quality.

High growth rates of lettuce can result in a calcium deficiency disorder called tipburn. Calcium is not phloem mobile, so most calcium is supplied through the xylem (Marchner, 1995). Rapidly growing meristems have poorly developed xylem to supply calcium. Furthermore, lettuce meristems can be located in the interior of older, more developed leaves thereby reducing transpiration and calcium supply (Barta and Tibbitts, 1986; Barta and Tibbitts, 1991; Barta and Tibbitts, 2000). The faster the growth rate, the more calcium is needed and the rate and extent of tipburn development increases. This same deficiency is analogous to tipburn in strawberry, sugar beets, and cabbage, and blossom end rot in tomatoes (Mostafa and Ulrich, 1976; Bradfield and Guttridge, 1984; Ho et al., 1993; Cubeta et al., 2000; Morard et al., 2000).

Controlled environments offer a way to optimize the environment to achieve the full genetic yield potential. Paradoxically, an optimized environment results in higher growth rates, and as a result, lettuce production in controlled environments is particularly sensitive to tipburn. Because of these issues, the limits of lettuce productivity have not been explored. Genetic selection for lettuce cultivars that are less susceptible to tipburn has resulted in tipburn resistance in the field (Welsh et al., 1983; Ryder and Waycott, 1998), but they have not been tested in controlled environment production. Management to deal with tipburn in controlled environments includes low light, low temperatures, and harvesting before the first sign of tipburn. These all make it impossible to know what the

limits of lettuce productivity are.

In this study, we optimize the environment for lettuce in controlled environments. We manipulate temperature and light to maximize lettuce quantity and quality without sacrificing growth rates. Three of the determinants of yield (radiation capture, quantum yield, and carbon use efficiency) are described to provide insight into what is most sensitive to altered temperatures. In doing so, insight can be gained as to the most limiting factors in lettuce production. This provides goals for breeders and managers to obtain maximum productivity from more typical lettuce growing conditions.

Materials and Methods

A total of three experiments were performed to explore the high light and temperature responses of lettuce. For convenience, a table is provided that summarizes the growth conditions of each trial (Table A-1).

Studies with Temperature

Lettuce (*Latuca sativa* L. cv. Grand Rapids) was germinated and transplanted after four days into small chambers at a density of 106 plants m^{-2}. Temperature treatments were arranged as randomized complete block design, and analyzed using linear regression. Treatments were initiated upon transplanting at were 23/18, 26/21, 29/24, 32/27, 35/30 C day/night.

A 10-chamber open gas-exchange system was used as described previously (van Iersal and Bugbee, 2000). Each chamber is $0.5 \times 0.4 \times 0.9$ m (L $\times$ W $\times$ H) and fully enclosed a hydroponic tub. Chamber temperature was controlled to within ±0.2C of set

point, and CO_2 controlled to within ±2% of set point of 1200 µmol mol^{-1}. CO_2 was

elevated to ensure that photosynthesis would be light- rather than CO_2 limited, to

optimize growth, and to eliminate photorespiration (Long, 1991; Bugbee et al., 1994).

Root-zone temperature was maintained by activating flexible heat-stripping wrapped

around the outside of the hydroponic tubs when the temperature fell below the average

daily temperature set point. Hydroponic solution was bubbled with the same CO_2-

enriched air as that used in the canopy. CO_2-gas-exchange of each of ten different whole

canopies was monitored once every 10 minutes. CO_2 measurements were made using

two infrared gas analyzers (LI-COR model 6251), one in absolute mode and one in

differential mode. A delta CO_2 was calculated using pre-CO_2 minus post-CO_2

concentrations, and photosynthetic and respiration rates were calculated by multiplying

the delta CO_2 by the flow rate (Mitchell, 1992; Bugbee, 1992).

The pH of the hydroponic solution was maintained between 4 and 5 in order to

eliminate CO_2 dissolved in solution (Monje and Bugbee, 1998). Relative humidity was

maintained between 60 and 85%. PPF was provided by water-filtered HPS lamps that

provided 600 µmol m^{-2} s^{-1} ± 30 µmol m^{-2} s^{-1} using a 16-h photoperiod to provide 35.6 mol

photons per m^2 per day. Side lighting was reduced by wrapping each chamber in a

reflective Mylar curtain and adjusted so that the top of the curtain was level with the top

of the canopy.

Ground cover was measured every other day using a digital camera positioned

directly above the canopies, and the images were processed for the ratio of leaf cover to

total area. Absorbed PPF was also measured with a line quantum sensor with 52 sensors

along a 43-cm length by measuring PPF incident, reflected, and transmitted through the

canopy (Klassen et al., 2000). Because of biases between the two methods of estimating

radiation capture, early digital images were used early to estimate radiation capture and

the line quantum sensor method was used when ground cover reached about 80%. We

could therefore accurately estimate the amount of light that was absorbed each day.

Canopy quantum yield (CQY) was then calculated by the following equation:

$$CQY = P_{gross} / \text{light absorbed} = \text{mol C fixed} / \text{mol photon absorbed}$$

P_{gross} for the day period (mol C m^{-2} d^{-1}) was divided by total photons absorbed (mol

photons m^{-2} d^{-1}) to give CQY (mol C fixed per mol photons absorbed). Gross

photosynthesis (P_{gross}) is a calculated term that incorporates both the net C fixed (P_{net}) and

the C that is simultaneously being respired. P_{gross} can be calculated as:

$$P_{gross} = P_{net} + R_{d}.$$

Net photosynthesis (P_{net}) and night-time respiration (R_{n}) were measured directly.

Since day-time respiration (R_{d}) can not be measured directly, P_{gross} is calculated as the

sum of P_{net} and some percentage of night-time respiration rate. Different studies have

indicated that R_{d} can remain high during the day due to higher carbohydrate content

during the day (Azcón-Bieto and Osmond, 1983), or can be lower due to some type of

light-inhibition of respiration (Atkin et al., 2000; Sharp et al., 1984). We have taken the

common approach to assume that R_{d} is occurring at the same rate as R_{n}. R_{d} would then be

defined as

$$R_{n} * (\text{time in light})/(\text{time in darkness})$$

so for a 16-h photoperiod, $R_{d} = R_{n} * 2$. In these equations, respiration assumes a positive

value (i.e. mass respired).

A combination of these parameters can be used to determine CUE in the following

manner:

$$CUE = P_{gross} / DCG$$

where DCG is daily carbon gain and is determined by subtracting R_n from P_{net}:

$$DCG = P_{net} - R_n.$$

Studies with Light

Both light studies were performed with lettuce seedlings sown directly into 12.7 ×

12.7 × 12.7 cm pots. Pots were filled with 1:1 peat perlite mixture irrigated twice daily

with nutrient solution. Soluble fertilizer (Peter's 5-11-26) was combined with reagent

grade $CaNO_3$ and Fe-EDDHA to give the following concentrations: 7.2 mM N, 0.75 mM

P, 2.7 mM K, and 20 μM Fe. Only 25% of the N was supplied with the mixed fertilizer

and of that, 1% was in the NH_4^+ form. This is important because NH_4^+ strongly inhibits

the uptake of calcium.

Seedlings in both studies were thinned two days after emergence to one plant per

pot. Four pots were grouped together to form a lettuce canopy. Side lighting was

controlled with a reflective Mylar barrier that was raised daily to match canopy height.

Light was from HPS lamps in a 16-h photoperiod. Four 10-cm fans were positioned in

each corner to increase the wind speed to an average of 0.68 m s^{-1} ± 0.18 m s^{-1} across the

chamber. Air temperatures were maintained at 30/25 C beginning at seeding based on the

results of our temperature study. Temperatures were measured with one shielded,

aspirated, type-E thermocouple per chamber. Leaf temperature was measured with an

precision infrared thermocouple that corrected for sensor body temperature (model IRT-P, Apogee Instruments Inc., Logan, UT). The sensor was positioned 8 to 10 cm from target leaf at a 60 angle to minimize self shading. Relative humidity was controlled at 75% during the day and night (model 50Y, Vaisala, Inc., Woburn, MA). CO_2 was elevated to 1200 μmol mol^{-1}.

In the initial light study, a single chamber was maintained at PPF of 500 μmol m^{-2} s^{-1} $\pm$ 25 μmol m^{-2} s^{-1} and another at 1000 μmol m^{-2} s^{-1} $\pm$ 50 μmol m^{-2} s^{-1}. PPF was measured with a line quantum sensor with 32 quantum sensors along a 27-cm length (model LQSV-ELEC, Apogee Instruments, Logan, UT) and adjusted once a week by adding neutral-density shade cloth or reflectors to the sides of the chamber. Two canopies each of four cultivars, 'Tiber', 'Waldmann's Green', 'Grand Rapids' and 'Buttercrunch' were used in both chambers. One canopy of each cultivar in both chambers had ~25% relative humidity air blown directly on the meristem at a rate of 1 L min^{-1} as was done in Goto and Takakura (1992a). Qualitative observations were made for each cultivar. Yield data was pooled across cultivars and analyzed with ANOVA and regression (SigmaStat, SPSS Science, Chicago, IL).

In the second light study, two canopies each of 'Waldmann's Green' and 'Buttercrunch' were grown in each chamber maintained at 1000 μmol m^{-2} s^{-1} $\pm$ 50 μmol m^{-2} s^{-1} of PPF at canopy height. Two canopies of each cultivar in both chambers had dry air blown directly on the meristem at a rate of 1 L min^{-1}. One chamber was harvested on day 19 (after imbibition) and the second chamber was harvested on day 28. This allowed us to see changes in final mass over the last few days of growth when the effects of

tipburn would be greatest. Data were kept separate between cultivars and analyzed with

ANOVA and regression. Ground cover was measured using a digital camera every other

day beginning at day seven until canopy closure.

Tipburn Index

Lettuce was scored after 28 days for severity of tipburn using a tipburn index that

took into account both severity of tipburn and number of affected plants. Many other

tipburn indices only take into account either tipburn severity or number of affected plants

(Misaghi et al., 1981a; Nagata and Stratton, 1994). As a result, a high score in those

indices do not discriminate between all plants having only minor tipburn or few plants

having major tipburn. In this index, the following equation was used:

$$\{[(S*5) + (M*3) + (L)] *100 \} / P * 5$$

where S is the number of plants with severe tip burn, M is the number of plants with

medium tipburn, L is the number of plants with light tipburn, and P is the total number of

plants. Severe tipburn is when most of the leaves have developed tipburn symptoms,

promoting plant core death, reduced growth, and would definitely be rejected

commercially. Medium tipburn symptoms exist when a few older leaves and the

meristem have symptoms. Light tipburn symptoms are when only the central leaflets

show any symptom of tipburn. In this index, there is more emphasis placed on severely

affected plants, and less emphasis on the plants with only minor tipburn symptoms. A

score of 100 would indicate that all plants have severe tipburn while a score of 20 may

indicate all plants have only minor symptoms.

Results and Discussion

Studies with Temperature

Lettuce growth rates differed substantially in the different temperatures (Figure A-1). The coolest and warmest average daily temperatures (20 and 33C, respectively) had the lowest final photosynthetic and respiration rates per unit ground area. The highest gas exchange rates were obtained in the treatments with average daily temperatures of 27 or 30C. The temperature at which lettuce is typically grown had intermediate photosynthesis and gas exchange rates. Tipburn was observed in all temperature treatments, but was not quantified for severity in this study.

Three of the determinants of yield are summarized in Figure 2. There was a strong influence of temperature on leaf expansion (Figure A-2A). The 27 and 30C treatments both reached near maximum PPF absorption by day 16. Conversely, the 33C treatment never reached that amount of PPF absorbed. Since radiation capture is a determinate of growth, the different magnitude of ground cover contributed to the different growth rates. Averaged over the 24 days after transplanting, the 27 and 30C treatments had the highest daily PPF absorbed (Figure A-2B). Total PPF absorbed is linearly related to the final dry mass (Figure A-3). The extrapolated line intercepting the x-axis should be an indication of the light compensation point for lettuce canopies. Assuming the 16-h photoperiod used in this study, the line suggests a light compensation point of 40 μmol m^{-2} s^{-1}, a value that is slightly higher than the 20 μmol m^{-2} s^{-1} estimated for "sun-plants"for C3 species (Taiz and Zeiger, 2002), but remarkably close considering the number was derived for an integrated calculation of total PPF absorbed.

The coolest and warmest temperature treatments had similar photosynthetic rates per unit ground area (Figure A-1), but had different rates of canopy closure with the 20C treatment reaching maximum absorption after 22 days and the 33C treatment expected to reach that level about 4 to 5 days later (extrapolating the line from Figure A-2A). This indicates that these two treatments differed in their photosynthetic capacity or photosynthesis per unit leaf area. Canopy quantum yield was lower for the coolest treatment and highest for the warmest treatment, but did not change significantly over time (Figure A-2C and A-2D). This pattern would not be expected in ambient CO_2 concentrations. This study was performed at elevated CO_2 (1200 µmol mol^{-1}), which effectively eliminated photorespiration.

Carbon use efficiency was stable after day nine and all temperature treatments had similar CUE of 0.6 after that point (Figure A-2E). There was no effect of temperature on CUE (Figure A-2F, slope = 0.0001). This finding is surprising given the widely accepted view that respiration rates increase exponentially in warmer temperatures. Similar CUE across temperatures indicates that respiration and photosynthesis increase in *exact proportion* to each other in elevated temperatures in a high CO_2 environment. Gifford (1995) also reported similar CUE in a wide range of species and growth conditions in ambient CO_2. Lettuce plants grown in elevated temperatures are equally efficient as lettuce grown in cool temperatures at conserving the carbon fixed during the day.

Chlorophyll content increased significantly with warmer temperatures (Figure A-4). The warmest treatment had about 10-fold higher chlorophyll content than the coolest treatment. This effect is especially interesting considering the relatively high light in this

study (600 μmol m^{-2} s^{-1}) and the belief that high light in lettuce will result in photobleaching. Our results indicate a strong effect of temperature in determining chlorophyll content rather than high light, per se.

Studies with Light

A slight flattening of the light response curve beyond 500 μmol m^{-2} s^{-1} was observed in the initial PPF response study (Figure A-5). Yield nevertheless increased beyond 500 μmol m^{-2} s^{-1}. Surprisingly, the literature on lettuce response to high PPF is not clear. There are some reports of lettuce shoot dry weight decreasing under high PPF (Tibbitts et al., 1983; Mitchell et al., 1991), while others report an increase with elevated PPF (Mitchell et al., 1997; Knight and Mitchell, 1983; Knight and Mitchell, 1988; Guadreau et al., 1994). Tipburn in our high-PPF canopies was more severe than in lower light, however, so *edible* yield likely was *not* increased with additional light.

There was a linear yield response to increased PPF in the canopies receiving air on the meristem during growth (Figure A-5). Tipburn was not observed in these canopies as well, suggesting that the flattening of yield in response to PPF is due to tipburn and a lack of continued leaf expansion in the latter stages of growth (after canopy closure) when growth rate is maximal. The differences between the aerated and unaerated was not significant, however (P = 0.084). There was more variation in the aerated treatment that we believe was independent of the treatment effect (e.g. side lighting), and some due to different cultivars not responding similarly to the aeration and PPF treatments, so in further studies, these issues were addressed by reducing the discrepancies in side lighting and reducing the number of cultivars tested to two from four. We also tested the

hypothesis that the differences in aerated versus unaerated canopies would manifest themselves late in canopy growth by harvested canopies both early (19 days) and later (28 days).

In the second PPF study, we observed no tipburn in the 'Waldmann's Green' and very little tipburn in the 'Buttercrunch' aerated canopies (Table A-1). This finding confirms earlier work that proved the concept of aerating meristems to reduce tipburn (Goto and Takakura, 1992a; Goto and Takakura, 1992b). However, this study differs from previous work in that we eliminated tipburn at extremely high PPF (1000 μmol m^{-2} s^{-1}) compared to the original studies that used 180 μmol m^{-2} s^{-1} and 230 μmol m^{-2} s^{-1}. Symptoms of tipburn initially appeared for the unaerated treatments on about day 14 for 'Buttercrunch' and about day 16 for 'Waldmann's Green'. This is consistent with previous results and suggests that 'Waldmann's Green' is somewhat resistant to tipburn (Bres and Weston, 1992).

Table A-1. Extent of tipburn on 'Buttercrunch' and 'Waldmann's Green' lettuce

cultivars with and without air blown on the meristems.

Cultivar	Treatment	Tipburn Index (%)
Buttercrunch	Air on meristem	5
	Control	95
Waldmann's Green	Air on meristem	0
	Control	60

There was no difference in the fresh mass between the aerated and unaerated

treatments of either cultivar at the first harvest (Figure A-6A). Over the next nine days,

however, both the treatments increased their mass significantly with a larger increase in

the aerated treatments. All aerated treatments had significantly higher fresh mass than the

unaerated canopies. There were no differences between cultivars.

There also were no differences in percent dry matter at the first harvest (Figure A-

6B). At the second harvest, however, all un-aerated canopies had significantly higher

percent dry matter. This finding confirmed our observations during harvest that the

aerated canopies were more succulent and fragile. As a result of differences in percent

dry matter, the final dry mass for both cultivars in each treatment were similar (Figure A-

6C). This was surprising to us for the following reasons: We believed that lack of tipburn

would result in greater leaf expansion for late-forming leaves and would therefore result

in higher yield (dry mass). We also believed that once tipburn began in the unaerated

controls, the canopies would not continue to grow at the same rate. Furthermore, we

assumed the differences in percent dry matter would be insignificant between the

treatments.

Canopy closure occurred on day 16 for both 'Buttercrunch' and 'Waldmann's Green' in both aerated and unaerated canopies. When canopy closure occurs in lettuce, approximately 80% of PPF is absorbed (Ritchie et al., unpublished data). In the following two to three days, light absorption is maximized at between 90 to 95%. Our assumption of greater leaf expansion of aerated canopies may have been true, but the amount of light absorbed for both treatments was already at or close to the maximum possible when tipburn began. Therefore, any potential differences in duration of leaf expansion would not necessarily result in differences in light capture.

Mature leaves in unaerated treatments were still green and presumed functional at the end of the study. Therefore, they were still able to accumulate mass regardless of young, tipburn affected leaves late in growth. The carbon fixed late in growth was partitioned differently in the two treatments (Figure A-7A). The aerated canopies had higher specific leaf area (SLA) than unaerated canopies so aeration maintained their ability to make thinner leaves for more efficient light capture. The shoot harvest index decreased from the initial harvest to the final harvest in both treatments, but decreased more in the unaerated controls (Figure A-7B). Since all leaf mass was counted as edible, the differences in SHI was a result of greater stem mass in unaerated controls.

While all leaf mass was counted as edible, the treatments differed greatly in quality of lettuce. No tipburn was observed in any of the 'Waldmann's Green' aerated canopies and only slight tipburn was observed in the aerated 'Buttercrunch' canopies. All unaerated 'Buttercrunch' canopies had severe tipburn with both interior and exterior malformed leaves, blackened meristem, and overall shorter, misshapen heads. Unaerated

'Waldmann's Green' also had tipburn in all unaerated canopies, but the severity was less than that of unaerated 'Buttercrunch'. Only the inner-most leaves were blackened and no outer leaves showed symptoms of tipburn. Because of the extent of tipburn, none of the harvested leaves for 'Buttercrunch' and only about 2/3 for 'Waldmann's Green' would be considered 'edible'. In this sense, aeration of the meristem can greatly increase yield of lettuce by eliminating tipburn.

Lettuce productivity was increased greatly with higher light and temperatures than typically used. High lettuce quality can be maintained if tipburn is eliminated. Aerating the meristem is not a practical solution for most situations. Shibata et al. (1995) reported a 30% increase in yield in a lettuce production facility when air was blown down vertically onto plants, but they did not report the amount of light used in the study. Eliminating tipburn in the present studies was done to test whether yield was light saturated or if other, indirect factors caused the yield saturation in light response curves. Since this evidence indicates yield can be increased with much higher light than previously though possible for lettuce, the importance of eliminating tipburn to obtain maximum yields either by environmental management or breeding is emphasized.

Some progress has been made in both of these areas. Several tipburn resistant cultivars have been developed for the field, but what works for the field does not necessarily work in controlled environments. Our present lack of understanding of the fundamental differences between field and controlled environments also makes it difficult to extrapolate from the field to the chamber and vice versa.

High light can increase tipburn by increasing transpiration from exposed leaves

more than increasing transpiration from meristem tissue (Saure, 1998). Furthermore, high light increases growth rates, which results in more need for Ca in the meristem. Humidity can also influence tipburn incidence through its effect on transpiration rates (Collier and Wurr, 1981; Collier and Tibbitts, 1984). Furthermore, low humidity at night may reduce full rehydration and turgor, and result in less Ca being forced to leaf tips and meristems (guttation). Salinity (or solution electrical conductivity) can also reduce night-time re-hydration and guttation (Feigin et al., 1991). Too much salt can lower soils osmotic potential and prevent adequate water uptake. Good nutrition is also important in tipburn prevention (Misaghi et al., 1981b; Rosen, 1990). Ca uptake is prevented by other cations, so fertilization with NH_4^+ can reduce Ca uptake. Wind also can reduce tipburn by reducing the vapor pressure deficit around a meristem thereby increasing transpiration potential (Shibata et al., 1995). Finally, temperature can also influence tipburn incidence both through its effect on vapor pressure deficits around the plants and influencing growth rates (Yanagi et al., 1983; Misaghi and Grogan, 1978). All of these environmental factors can be controlled within chambers. However optimizing all of these parameters still does not eliminate tipburn. Slowing down growth rates does reduce tipburn, but when the goals are fast, optimized production, this management scenario will not work. Fortunately, in these situations (as in Advanced Life Support Systems for NASA or in so called lettuce factories), it may be practical to use hoses blowing air directly on the meristem in high light to maximize yields.

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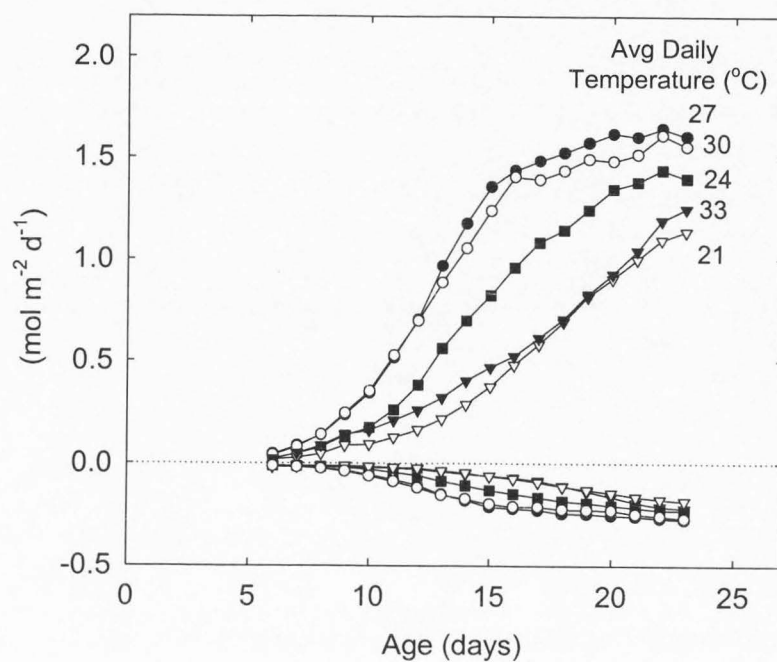
Figure A-1. Photosynthesis and respiration rates for lettuce canopies grown in 1200 µmol mol^{-1} CO$_2$ with constant day/night temperatures.

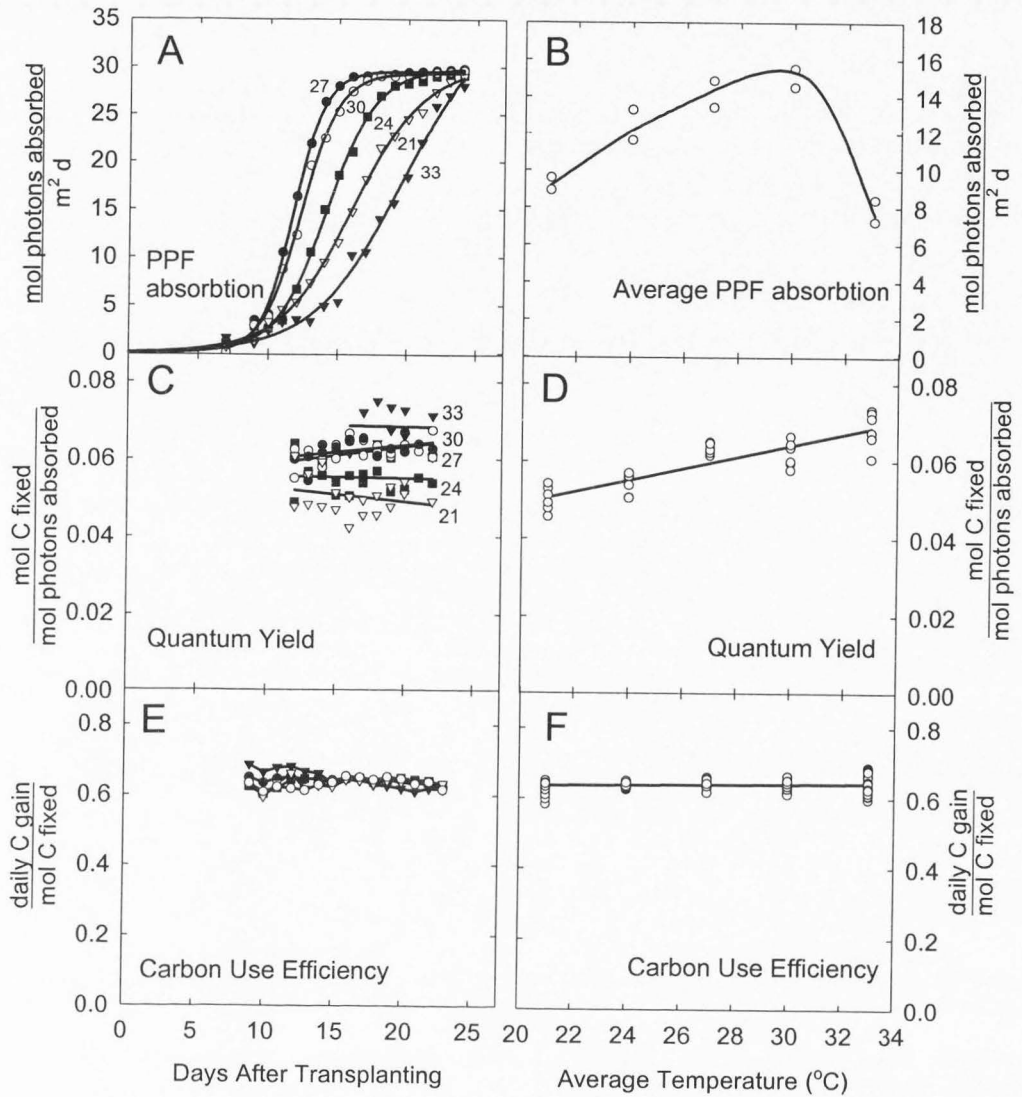Figure A-2. Three determinants of yield expressed on both a time and a temperature basis for lettuce canopies grown in 1200 μmol mol^{-1} CO$_2$ with constant day/night temperatures.
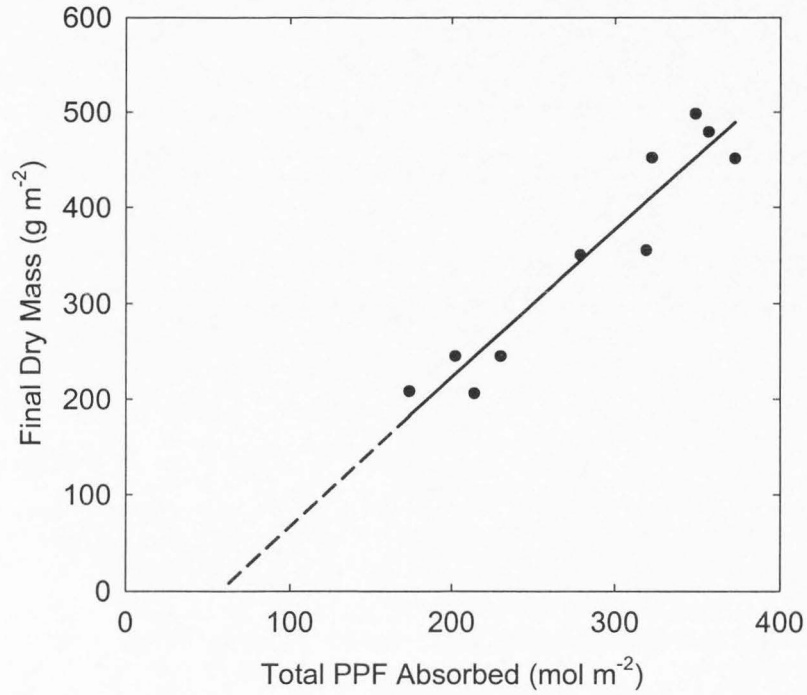
Figure A-3. Relationship between moles of photons absorbed and final dry mass. The relationship is linear, and if the regression line is extrapolated, an estimate for the canopy compensation point is obtained of about 60 mol m^{-2}.
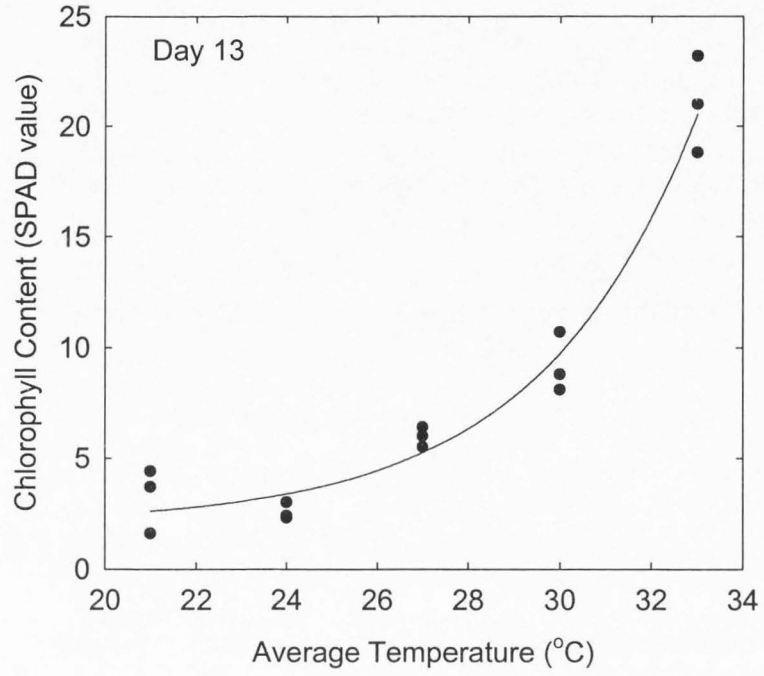
Figure A-4. Influence of temperature on chlorophyll content on day 13 as measured with a SPAD meter.
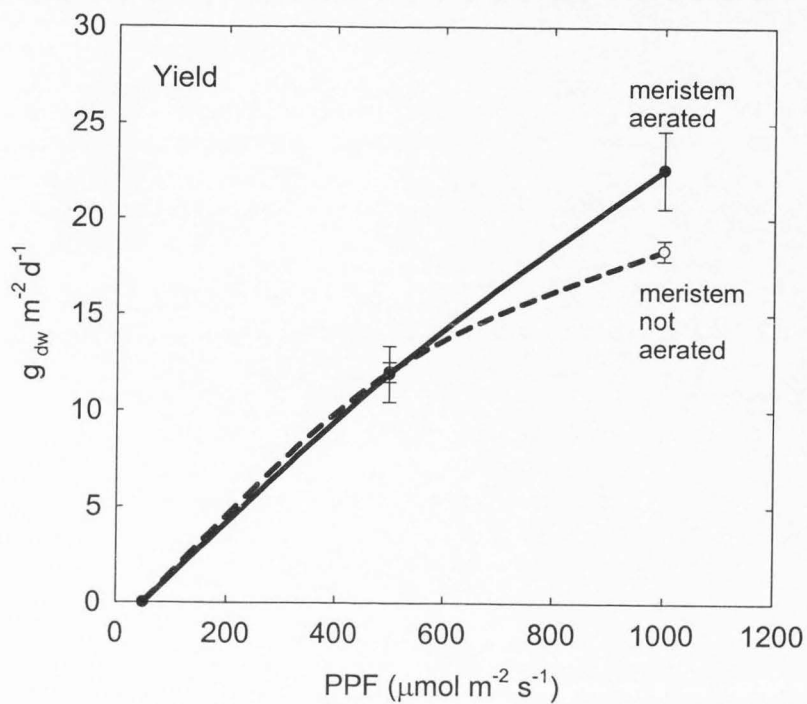
Figure A-5. Yield response of 'Waldmann's Green' and 'Buttercrunch' lettuce to high light with and without aeration on meristem. Data were pooled between cultivars and error bars are ± 1 standard error of the mean.
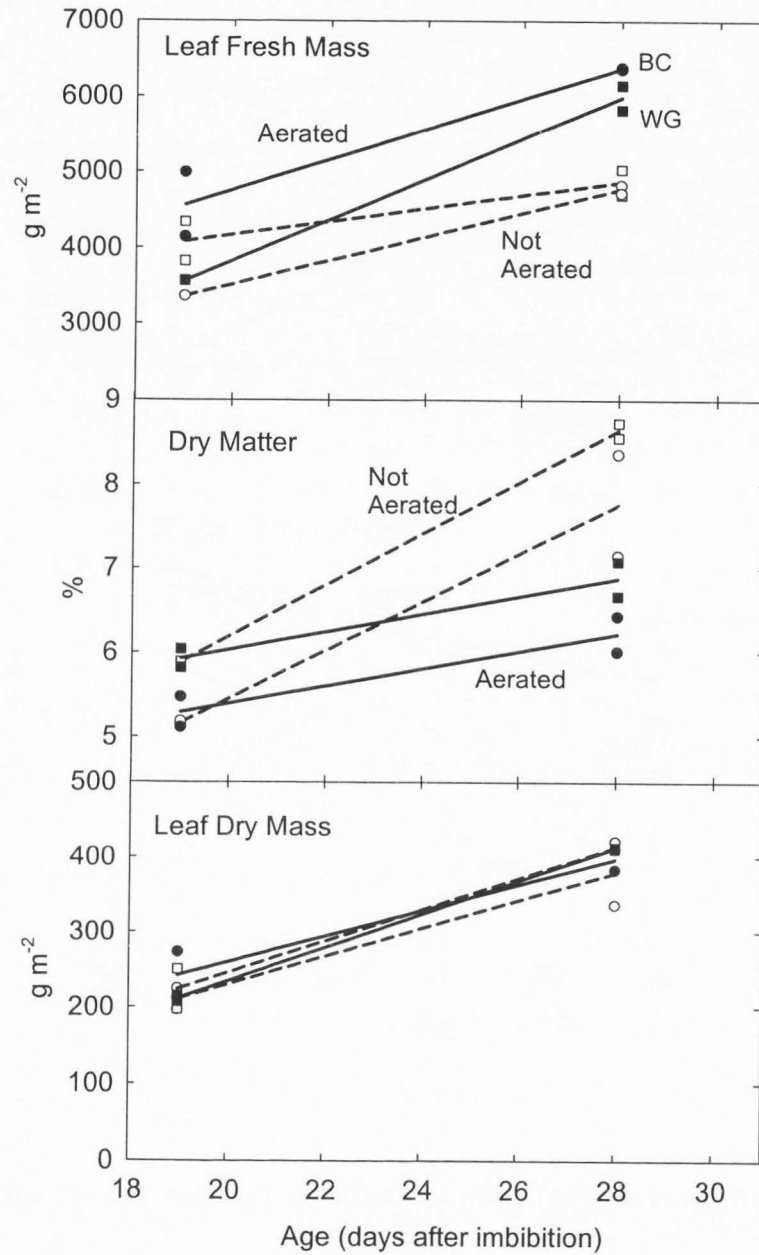
Figure A-6. Fresh mass, fraction of dry matter, and dry mass in 'Buttercrunch' (BC) and 'Waldmann's Green' (WG) with and without aeration during the final 8 days of growth. Most of the aeration effect occurred during the final week of growth.
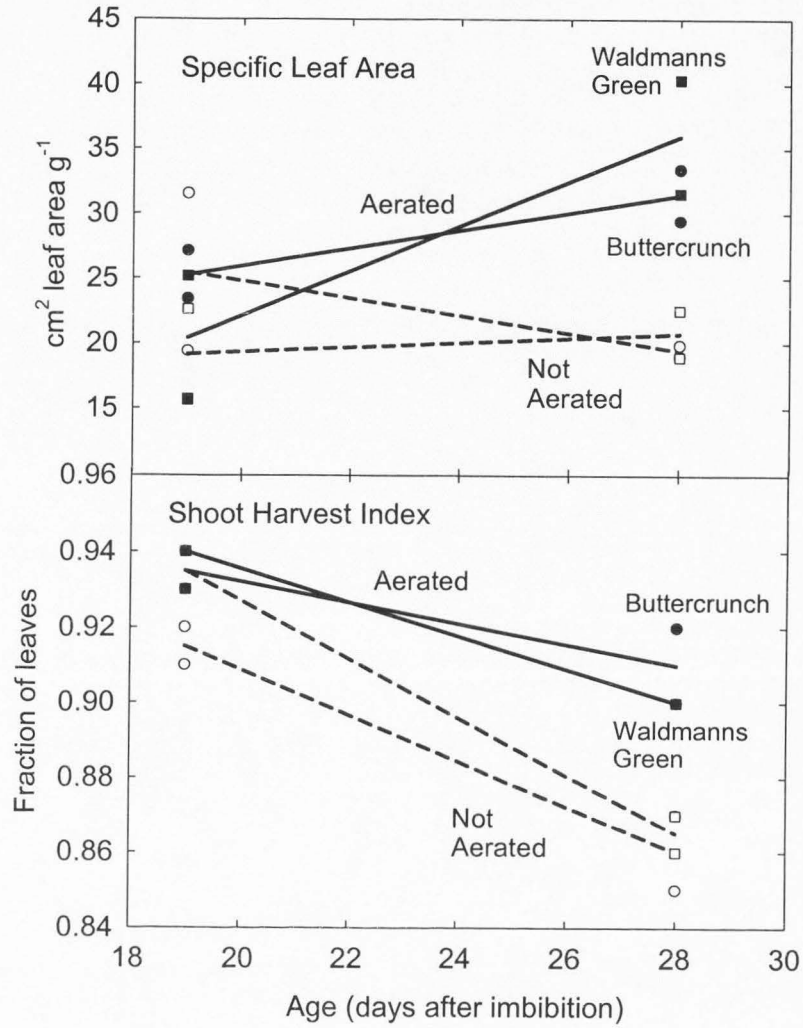
Figure A-7. Specific leaf area and shoot harvest index for both treatments and cultivars.

All leaves, regardless of their tipburn index were counted as edible for this analysis.

CURRICULUM VITAE

Jonathan Maddry Frantz

EDUCATION

Ph.D. in Plant Science, February, 2003.
Utah State University

M.S. in Horticulture, May, 1999.
Purdue University

B.S. in Biochemistry, May, 1996. (Minors in Chemistry and Biology)
Virginia Polytechnic Institute and State University

TEACHING EXPERIENCE

— *Utah State University* —

Greenhouse Management / Crop Production   Spring 2002
Guest lecture for Craig Aston in PLSC 3050
Topic: Plant lighting needs and measurement

Plant Nutrition   Fall 2001
Taught half a semester of PSB 5430/6430
Topics: Root growth, rhizosphere environment, symbiosis, and biochemical role of each
mineral nutrient.

Crop Physiology   Spring 2000, 2001, 2002, 2003
Guest lecture for Dr. Bruce Bugbee in PSB 5210/6210
Topics: Energy balance models, growth analysis, and crop respiration

Crop Physiology Laboratory   Spring 1999, 2000, 2001
Graduate teaching assistant for Dr. Bruce Bugbee in PSB 5210/6210
Taught Spring, 2003
Topics: Measuring radiation, photobiology, growth analysis, transpiration, open and closed system gas-exchange

— *Purdue University* —

Horticultural Production Technologies   Fall 1997
Guest lecture for Dr. Steve Weller in HORT 401
Topic: Controlled environment agriculture.

Horticultural Production Technologies   Fall 1997
Graduate teaching assistant for Dr. Steve Weller in HORT 401
Topic: growth structures, growth media, irrigation, supplemental lighting, pruning, fertilization, crop production, mechanization, and post-harvest handling.

AWARDS / MEMBERSHIPS / HONORS
— Awards —

American Society for Gravitational and Space Biology meeting; November 2001
   Third Place: Graduate Poster Competition
NCR-101 / UK Controlled Environment Users Group meeting; September 2001
   Graduate Student Travel Award
NASA Graduate Student Research Program Fellowship; July 2000 to present
Vice-Presidential Fellowship at Utah State University, Spring 1999
American Society for Gravitational and Space Biology meeting, 1998
   Honorable Mention: Graduate Poster Competition

— Memberships —

American Association for the Advancement of Science 2001 to present
American Agronomy and Crop Science Society of America 1999 to present
American Society of Horticultural Science 1996 to present

— Honors —

Graduated Cum Laude from VT, May, 1996.
Outstanding Undergraduate Student Award in the Biochemistry Department at VT,
   May, 1996
Dean's List all semesters at VT, 1992 to 1996.
Phi Kappa Phi 1994 to present
Alpha Phi Omega 1993 to 1996

INVITED TALKS

National Onion Growers Association summer meeting in Ogden, UT, July 18, 2002.
   "What's Next?...Onions in Space." *In place of B. Bugbee.*
Space Botany Meeting in Beijing, China, March 21 to 31, 2002. "Breeding and Selecting
   Plants for Use in Space" and "NASA funded research at Utah State University." *Part
   of seminar with B. Bugbee.*
NASA Johnson Space Center, July 12, 2000. "Respiration and Carbon Use Efficiency in
   Crop Plants."*Co-presenter with B. Bugbee.*

PUBLICATIONS
— Refereed publications —

Klassen, S. P., G. Ritchie, J. Frantz, D. Pinnock, and B. Bugbee. 2003. Real-time
   imaging of ground cover: relationships with radiation capture, canopy
   photosynthesis, and daily growth rate. In: Digital Imaging Techniques in
   Agriculture, A Special Publication of the Crop Science Society of America (CSSA).
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Frantz, J. M. and Bugbee, B. 2002. Anaerobic conditions improve germination of a
   gibberellic acid deficient rice. Crop Sci. 42:651-654.
Frantz, J. M., Joly, R. J. and Mitchell, C. A. 2001. Intracanopy lighting reduces
   electrical energy utilization by closed cowpea stands. Life Support and Biosphere
   Sci. 7:283-290.

Welbaum, G. E., Frantz, J. M., Gunatilaka, M. K., and Shen, Z. 2001. A comparison of the growth, establishment, and maturity of direct-seeded and transplanted sh_2 sweet corn. HortSci. 36:687-690.

Frantz, J. M., Joly, R. J., and Mitchell, C. A. 2000. Intracanopy lighting influences radiation capture, productivity, and leaf senescence in cowpea canopies. J. of Amer. Soc. Hort. Sci. 125:694-701.

Frantz, J. M., Frick, J., and Mitchell, C. A. 1999. A solid-matrix/liquid hybrid hydroponic system for establishment of small-seeded crop species. HortTech. 9:165-168.

Frantz, J. M., Chun, C., Joly, R. J., and Mitchell, C. A. 1998. Intracanopy lighting of cowpea canopies in controlled environments. Life Support and Biosphere Sci. 5:183-189.

Frantz, J. M., and Welbaum, G. E. 1998. Producing horticultural crops using hydroponic tobacco transplant systems. HortTech. 8:392-395.

Frantz, J. M., and Welbaum, G. E. 1998. Comparison of cabbage seedling growth in four transplant production systems. HortSci. 33:976-979.

— Non-Refereed Publications / Conference Proceedings —

Gunatilaka, M. K., Frantz, J. M., and Welbaum, G. E. 1997. Growing SH2 sweet corn from transplants. 5th International Symposium on Stand Establishment, Conference Proceedings. June 1-4, 1997, Columbus, Ohio. pp. 249-253.

Frantz, J. M., and Welbaum, G. E. 1995. A comparison of four cabbage transplant production systems. 3rd International Symposium on Stand Establishment, Conference Proceedings. April 23-26, Monterey, California. pp. 169-174.

Frantz, J. M., and Welbaum, G. E. 1995. Growing SH2 sweet corn from transplants. Proceedings of the 3rd International Symposium on Stand Establishment, Conference Proceedings. April 23-26, Monterey, California. pp. 199-202.