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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

Jonathan M. Frantz

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

of

DOCTOR OF PHILOSOPHY

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UTAH STATE UNIVERSITY
Logan, Utah

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ABSTRACT

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

by

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Utah State University, 2003

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Department: Plants, Soils, and Biometeorology

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 CO₂ 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 32°C.

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.
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.

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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 mainly 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 \textit{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-Beto 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-Beto 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 O₂ consumption and/or CO₂ generation were measured for short (minutes to hours) periods of time. The rate of respiration was strongly correlated to the rate of prior CO₂ 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 40°C. Respiration rates generally plateau at the next 5 to 10°C, 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 10°C 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 10°C 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 a 5C day/night difference in temperature across a range of 21C to 35C.

2. The role of substrate availability

Hypothesis 5: Elevated CO₂ will increase carbohydrate supply and decrease temperature sensitivity, whereas ambient CO₂ 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.

References


Daily Carbon Gain = area A - area B
Gross Photosynthesis = area A + area C
Carbon Use Efficiency = \( \frac{(\text{area A} - \text{area B})}{(\text{area A} + \text{area C})} \)

Figure 1-1. Diagram for calculating carbon use efficiency from a single day’s CO₂ gas-exchange data.
CHAPTER 2

NIGHT TEMPERATURE HAS A MINIMAL EFFECT ON RESPIRATION AND GROWTH IN RAPIDLY GROWING PLANTS

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 10°C 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 34°C in young, rapidly growing plant communities for up to 20 days. The day temperature was maintained at 25°C. CO₂ 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 10°C 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.

—

¹Coauthored 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 ($P_{\text{gross}}$) (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 (*Lactuca 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
m², tomato at 80 plants m², and soybean at 35 plants m².

Night temperatures were controlled across a 17°C range from 17 to 34°C. 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 (±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). CO₂ was controlled at 1,200 μmol mol⁻¹ for the duration of all the studies. These studies were conducted at elevated CO₂ 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 CO₂. 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 CO₂. 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 CO₂ 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 \( P_{\text{net}} \) (net photosynthesis, mol C m\(^{-2}\) ground \( \cdot \) d\(^{-1}\)) and \( R_n \) (night-time respiration, mol C m\(^{-2}\) ground \( \cdot \) night\(^{-1}\)), daily carbon gain (DCG) can be calculated as:

\[
\text{DCG} = P_{\text{net}} - R_n
\]

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

\( P_{\text{gross}} \) is a calculated term that reflects the net C fixed (\( P_{\text{net}} \)) and the amount of C that is simultaneously being respired. Because day-time respiration (\( R_d \)) can not be measured directly, \( P_{\text{gross}} \) is expressed as the sum of \( P_{\text{net}} \) 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 $R_n$ ($\mu$mol 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 $R_n$. In a 16-h light / 8-h dark photoperiod, $R_d = R_n \times 2$.

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:

$$(\text{posttreatment}_a \text{ day}_b / \text{pretreatment}_a \text{ value}) \div (\text{posttreatment}_{control} \text{ day}_b / \text{pretreatment value}_{control}) \times 100(\%)$$

where posttreatment$_a$ indicates the posttreatment value of parameter a (i.e., CUE, $P_{net}$, $R_{dark}$), 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 \( P_{\text{gross}} \), \( Q_{10} \) 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:

\[
Q_{10} = e^{\frac{(R_{nT} - R_{n \text{control}})}{(T - \text{control} / 10)}}
\]

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 \( \left( \frac{\text{slope a} - \text{slope b} - 0}{\text{variance of slope a}} \right) = t \) (degrees of freedom of slope a) (Neter et al., 1996).

**Results**

**Lettuce**

Night respiration increased 2.0% per degree C \( (F = 440.0, \text{df} = 9, P < 0.0001; \)
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 \( (F = 0.41, \text{df} = 9, P = 0.54; \text{Figure } 2-3B) \). 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 \( (F = 31.5, \text{df} = 9, P = 0.0005; \text{Figure } 2-3C) \). The sensitivity of CUE to temperature did not change during the 13 days of treatment based on comparison of slopes \( (P = 0.35, \text{df} = 8) \). There was no difference in final dry mass \( (F = 1.19, \text{df} = 9, P = 0.31) \) or in CCG \( (F = 0.23, \text{df} = 9, P = 0.64) \) after treatments were imposed (Table 2-1).

**Tomato**

Night respiration increased 2.7% per degree C with warmer nights \( (F = 185.2, \text{df} = 9, P < 0.0001; \text{Figure } 2-4A \text{ and } 2-5A) \). This caused the CUE to be slightly higher with cooler nights and lower with warmer nights \( (F = 35.8, \text{df} = 9, P < 0.0003; \text{Figure } 2-4B \text{ and } 2-5C) \). After 20 days of temperature treatment, there was no difference in the sensitivity of CUE or respiration to altered night temperature \( (P = 0.48, \text{df} = 8) \). Net photosynthesis was not affected by the altered night temperatures \( (F = 0.47, \text{df} = 9, P = 0.57; \text{Figure } 2-5B) \). There were no differences in the final dry mass \( (F = 0.43, \text{df} = 9, P = 0.53) \) of the treatments or in CCG \( (F = 0.28, \text{df} = 9, P = 0.61) \) 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 10°C rise in temperature between 10 and 30°C (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 $Y_g$) 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 20°C compared to 30/10°C 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 CO₂ 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 CO₂, 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 30°C. The day-time respiration would need to decrease by 75% for CUE to remain the same. There was no effect of temperature on $P_{\text{net}}$ so this should result in a 12% decrease in $P_{\text{gross}}$. 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) (Thomley 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-Menten 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 CO₂ (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 Q₁₀ 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 m² ground area), cumulative 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 ($P>0.59$).

<table>
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<tr>
<th>Crop</th>
<th>Night temp.</th>
<th>Final plant mass (g m⁻²)</th>
<th>CCG after treatment initiation (mol C m⁻²)</th>
<th>Sensitivity to temperature ($Q_{10}$)</th>
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<td>490.5</td>
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<tr>
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<td>1.38 to 1.34</td>
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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.
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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 (25°C in lettuce and tomato, and 20°C in soybean).
CHAPTER 3
RADIATION CAPTURE, PHOTOSYNTHESIS, AND RESPIRATION AS AFFECTED BY TEMPERATURE AND CO₂

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, CO₂ concentration may influence the effect of temperature. Using whole canopy CO₂ gas exchange, four trials were run to test the effect of constant temperature, or a 5°C day/night temperature difference at two CO₂ concentrations (ambient or 400 µmol mol⁻¹ and elevated or 1,200 µmol mol⁻¹) in lettuce (Lactuca sativa) communities. Temperature greatly influenced the rate of leaf emergence and expansion, which resulted in different levels of radiation capture. Concentration of CO₂ had a minor effect on radiation capture, with canopies grown in higher CO₂ concentrations reaching canopy closure about three days earlier than ambient CO₂-grown canopies. Canopy photosynthetic efficiency (quantum yield) decreased with increasing temperature in ambient CO₂ canopies, but increased with temperature in elevated CO₂. Carbon use efficiency was not affected by temperature in elevated CO₂, but decreased at the highest temperature in low CO₂. 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.

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 20°C day/night temperature was slightly improved compared to a canopy grown at 30°C days and 10°C 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 31°C (Reddy et al., 1993), while broccoli (*Brassica oleracea* L. *italica*) has an optimum of only 21°C (Olesen and Grevsen, 1997). Lettuce (*Lactuca 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 23°C day and 7 to 11°C 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 CO₂ with available light. Photorespiration, a competing reaction that fixes O₂ instead of CO₂, increases with temperature for C₃ crops, and reduces QY in warmer temperatures in ambient CO₂. Photorespiration can be significantly reduced at [CO₂] of 1,200 µmol mol⁻¹ so that electron transport becomes the rate limiting factor in CO₂ fixation. The potential rate of electron transport continues to increase up to 30 to 35°C (Farquhar et al., 1980). Therefore, the temperature optimum for QY of C₃ species should increase several degrees under elevated CO₂ (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 ($P_{\text{gross}}$) (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 25°C. 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 (*Lactuca 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 35°C, and two trials were conducted with different day/night temperatures of 21/18, 26/21, 29/24, 32/27, and 35/30°C. 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 ±0.2°C of the set point, and root temperatures were within ±0.5°C 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 CO₂ exchange rates of $P_{\text{net}}$ (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:

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

$P_{\text{gross}}$ is a calculated term that reflects the net C fixed ($P_{\text{net}}$) and the amount of C that is simultaneously being respired. Because day-time respiration ($R_d$) can not be measured directly, $P_{\text{gross}}$ is expressed as the sum of $P_{\text{net}}$ 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 $R_n$ ($\mu$mol m\text{ground}⁻² s⁻¹) are equal when temperatures are constant. In a 12-h photoperiod, $R_d$ (mol m\text{ground}⁻² d⁻¹) then equals $R_n$. In a 16-h light / 8-h dark photoperiod, $R_d = R_n \times 2$. In these equations, respiration assumes a positive value (i.e., mass respired). $P_{\text{gross}}$ (mol C m\text{ground}⁻² d⁻¹) can, therefore, be calculated as:

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

CUE is the ratio of carbon gained per day to total carbon fixed, or:
\[ \text{CUE} = \frac{\text{DCG}}{\text{P}_{\text{gross}}} \]

Sensitivity analysis of CUE to changes in \( R_d \) to calculate \( P_{\text{gross}} \) 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 \( P_{\text{gross}} \), \( Q_{10} \) 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 7°C 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 \( \mu \text{mol mol}^{-1} \) \( \text{CO}_2 \), Constant Temperature

Data from both 32.5°C treatment and one of the 35°C 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 CO₂, 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 CO₂ 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 CO₂ 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 35/30C 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 CO₂, 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 ($\pm 0.017$ 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 nighttime 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 CO₂ 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 CO₂ was not believed to lead to better water relations and improved leaf expansion. High CO₂ 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 CO₂ and was lower in ambient CO₂, 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 CO₂ in lower light. Photorespiration should increase with an increase of temperature from 20°C to 35°C in ambient CO₂, causing QY to decrease from about 0.065 to about 0.057 (Farquhar et al., 1980; Long, 1991). In the ambient CO₂ 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 Rubisco should increase with temperature, electron transport would increase and peak about 30 to 35°C (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) (Thomley 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-Menten 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.

References


Figure 3-1. Photosynthesis and respiration rates for all temperature and CO₂ 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 CO₂ 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 CO₂ 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 CO₂ with a 5°C 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 CO₂ with a 5°C difference in day/night temperatures.
CHAPTER 4
ACCLIMATION TO SHADE: PHOTOSYNTHESIS, RESPIRATION, AND CARBON USE EFFICIENCY

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 (*Lactuca 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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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 (*Lactuca 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 (*Lactuca 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 25°C day/night temperature including the roots. Chamber temperature was controlled to within ±0.2°C 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 CO₂-enriched air as that used in the canopy. The CO₂-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 CO₂. 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 CO₂ 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 (±5%) for lettuce and 650 µmol m⁻² s⁻¹ (±5%) for tomato. Both trials were run using a 16-h photoperiod to provide 35.6 mol photons per m² per day for lettuce and 37.4 mol photons per m² 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) positioned 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:

\[
CUE = \frac{DCG}{P_{\text{gross}}}
\]

where \(P_{\text{gross}}\) is gross photosynthesis and DCG is daily carbon gain. Using the measured CO₂ exchange rates of \(P_{\text{net}}\) (net photosynthesis, mol C m⁻² d⁻¹) and \(R_{\text{n}}\) (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:

\[
DCG = P_{\text{net}} - R_{\text{n}}
\]

where \(P_{\text{gross}}\) is a calculated term that incorporates both the net C fixed (\(P_{\text{net}}\)) and the C that is simultaneously being respired. Because day-time respiration (\(R_{d}\)) can not be measured
directly, $P_{\text{gross}}$ is calculated as the sum of $P_{\text{net}}$ 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 $R_n$ ($\mu$mol m$_{\text{ground}}^{-2}$ s$^{-1}$) are equal when temperatures are constant. In a 12-h photoperiod, $R_d$ (mol m$_{\text{ground}}^{-2}$ d$^{-1}$) then equals $R_n$. In a 16-h light/8-h dark photoperiod, $R_d = R_n \times 2$. In these equations, respiration assumes a positive value (i.e., mass respired). $P_{\text{gross}}$ can, therefore, be calculated as:

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

Sensitivity analysis of CUE to the assumption of $R_d$ to calculate $P_{\text{gross}}$ 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:
(Posttreatment\textsubscript{a} day\textsubscript{b} / Pretreatment\textsubscript{a} value) ÷ (Posttreatment\textsubscript{control} day\textsubscript{b} / pretreatment value\textsubscript{control})

where posttreatment\textsubscript{a} indicates the posttreatment value of parameter a (i.e., CUE, $P_{\text{net}}$, and $R_{\text{n}}$), day\textsubscript{b} indicates the day after treatment, pretreatment\textsubscript{a} value is the value of the parameter of interest on the day before treatments began, posttreatment\textsubscript{control} day\textsubscript{b} is the posttreatment value of the parameter of interest on the same posttreatment day, and pretreatment value\textsubscript{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 $P_{\text{gross}}$ for the day period (mol C m\textsuperscript{-2} d\textsuperscript{-1}) was then divided by total photons absorbed (mol photons m\textsuperscript{-2} d\textsuperscript{-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 $P_{\text{net}}$ decreased immediately after applying the shade treatments with a 76% decrease occurring in PPF of 150 $\mu$mol m\textsuperscript{-2} s\textsuperscript{-1}. Values of $P_{\text{net}}$ 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 $P_{\text{net}}$ in both species. For both species, we observed a near 1:1 ratio in amount of shade in relation to reduced $P_{\text{net}}$. 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:

$$\frac{1}{\text{CUE}} = 1 + r_g + r_m \times \frac{1}{\text{RGR}}$$
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 Thomley 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 Rubisco 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.

References


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 CO₂ 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

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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*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 CO₂ 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, $P_{\text{gross}}$). Because it is a ratio, it is less sensitive to a change in either DCG or $P_{\text{gross}}$; 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 $\mu$mol m$^{-2}$ s$^{-1}$ for the duration of the trial), two with low PPF (80 $\mu$mol m$^{-2}$ s$^{-1}$) or high PPF (about 600 $\mu$mol m$^{-2}$ s$^{-1}$). Slopes of lines were compared to see if their slopes were equal using the test statistic 

\[
\frac{(\text{slope}_a - \text{slope}_b) - 0}{\text{variance of slope}_a} = t_{\text{degrees of freedom of slope}_a} \quad (\text{Neter et al., 1996}).
\]

**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 $\mu$mol 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 CO₂. 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 CO₂ 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 (±5%) for all chambers until canopy closure. Both trials were run using a 16-h photoperiod to initially provide 17.28 mol photons per m² per day.

Shade was applied two days after canopies closed, which occurred 16 days after transplanting for the canopies at 30°C and 24 days after transplanting for the canopies at 20°C. 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 = \frac{DCG}{P_{\text{gross}}}

where DCG is daily carbon gain and \( P_{\text{gross}} \) is gross photosynthesis. Using the measured \( \text{CO}_2 \) exchange rates of \( P_{\text{net}} \) (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:

\[
\text{DCG} = P_{\text{net}} - R_n
\]

where \( P_{\text{net}} \) is net photosynthesis and \( R_n \) is night respiration. Gross photosynthesis (\( P_{\text{gross}} \)) is a calculated term that incorporates both the net C fixed (\( P_{\text{net}} \)) and the C that is simultaneously being respired. Because day-time respiration (\( R_d \)) can not be measured directly, \( P_{\text{gross}} \) is calculated as the sum of \( P_{\text{net}} \) 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:

\[
R_n * \frac{(\text{time in light})}{(\text{time in darkness})}.
\]

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, \( P_{\text{gross}} \) can be calculated as:

\[
P_{\text{gross}} = P_{\text{net}} + R_d.
\]

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 $P_{\text{gross}}$ 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:

$$\frac{(\text{Posttreatment}_a \text{ day}_b \ / \ \text{Pretreatment}_a \text{ value}) \div (\text{Posttreatment}_\text{control} \text{ day}_b \ / \ \text{pretreatment value}_\text{control})}{(\text{Posttreatment}_a \text{ day}_b \ / \ \text{Pretreatment}_a \text{ value}) \div (\text{Posttreatment}_\text{control} \text{ day}_b \ / \ \text{pretreatment value}_\text{control})}$$

where posttreatment$_a$ indicates the posttreatment value of parameter a (i.e., CUE, $P_{\text{net}}$, 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$_\text{control}$ day$_b$ is the posttreatment value of the parameter of interest on the same posttreatment day, and pretreatment value$_\text{control}$ 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 a forced-air oven at 80°C 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 Al₂(SO₄)₃. The tissue and solution were shaken four times during the 1-h extraction period. The solution was measured with a NO₃⁻ selective and an associated reference electrode (Model 930700 and Model 900200 Thermo Orion, Beverly, MA). The readings were then converted from volts to NO₃⁻-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 \( W \):

\[ \frac{R}{W} = cW/W + mG/W \]

the equation simplifies to:

\[ \text{specific respiration} = c + m \times \text{RGR} \]
or specific respiration, which is a linear function of relative growth rate (RGR) times the growth coefficient plus the maintenance coefficient. Using CO₂ 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.

**30°C 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
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).

20°C 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 \( (F = 226.1, \, df = 23, \, P < 0.001, \, r^2 \text{ of at least } 0.91) \) (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 \((80 \, \mu\text{mol m}^{-2} \text{ s}^{-1})\) and high \((600 \, \mu\text{mol m}^{-2} \text{ s}^{-1})\) 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 $\text{NO}_3^-$ content, while low $\text{NO}_3^-$ content was observed at high PPF. Stem $\text{NO}_3^-$ 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 CO₂, which reportedly causes reductions in maintenance requirements, due in part to decreased Rubisco concentrations of plants grown in elevated CO₂ (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 NO₃⁻ 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 NO₃⁻ N may indicate a smaller requirement of maintenance respiration, possibly maintaining ion gradients rather than functional proteins.

Surprisingly little difference in N or NO₃⁻ content was observed between the two temperature environments. Kafkafi (1990) reported about a 33% increase in leaf NO₃⁻ concentration as temperature increased from 24 to 34C and about a 50% decrease in root NO₃⁻ as the temperature increased from 24 to 34C. This was attributed to less NO₃⁻ 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.

References


Kafkafi, U. 1990. Root temperature, concentration, and the ratio NO₃⁻ / NH₄⁺ effect on


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.

<table>
<thead>
<tr>
<th></th>
<th>PPF (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Temperature (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80</td>
<td>300</td>
</tr>
<tr>
<td><strong>Growth (mol C$<em>{\text{resp}}$ mol C$</em>{\text{growth}}$)$^{-1}$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.587</td>
<td>0.506</td>
</tr>
<tr>
<td>Std. error</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 18.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.009</td>
<td></td>
</tr>
<tr>
<td><strong>Maintenance (mmol C mol C d$^{-1}$)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.39</td>
<td>6.47</td>
</tr>
<tr>
<td>Std. error</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 10.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.024</td>
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</tr>
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</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Leaf PPF (μmol m$^{-2}$ s$^{-1}$)</th>
<th>Stem PPF (μmol m$^{-2}$ s$^{-1}$)</th>
<th>Root PPF (μmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80</td>
<td>300</td>
<td>600</td>
</tr>
<tr>
<td>C</td>
<td>351</td>
<td>365</td>
<td>389</td>
</tr>
<tr>
<td>Std. error</td>
<td>2.2</td>
<td>3.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 80.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td>63</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td>Std. error</td>
<td>1.2</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 96.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>23</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Std. error</td>
<td>1.2</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 32.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.003</td>
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</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Leaf 20C</th>
<th>Leaf 30C</th>
<th>Stem 20C</th>
<th>Stem 30C</th>
<th>Root 20C</th>
<th>Root 30C</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>353</td>
<td>383</td>
<td>358</td>
<td>359</td>
<td>380</td>
<td>368</td>
</tr>
<tr>
<td>Std. error</td>
<td>2.0</td>
<td>2.0</td>
<td>6.9</td>
<td>6.9</td>
<td>3.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 108.0</td>
<td>P &lt; 0.001</td>
<td>F = 0.011</td>
<td>P = 0.923</td>
<td>F = 6.579</td>
<td>P = 0.083</td>
</tr>
<tr>
<td>Total N</td>
<td>49</td>
<td>52</td>
<td>30</td>
<td>32</td>
<td>42</td>
<td>45</td>
</tr>
<tr>
<td>Std. error</td>
<td>1.1</td>
<td>1.1</td>
<td>2.3</td>
<td>2.3</td>
<td>4.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 4.35</td>
<td>P = 0.11</td>
<td>F = 0.56</td>
<td>P = 0.50</td>
<td>F = 18.4</td>
<td>P = 0.023</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>16</td>
<td>15</td>
<td>15</td>
<td>18</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Std. error</td>
<td>1.1</td>
<td>1.1</td>
<td>2.0</td>
<td>1.7</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 0.612</td>
<td>P = 0.48</td>
<td>F = 1.29</td>
<td>P = 0.34</td>
<td>F = 3.14</td>
<td>P = 0.18</td>
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</table>
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.

<table>
<thead>
<tr>
<th>PPF (µmol m$^2$ s$^{-1}$)</th>
<th>Temperature (°C)</th>
<th>Quantum yield</th>
<th>Std. error</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>80</td>
<td>300</td>
<td>600</td>
<td>20</td>
</tr>
<tr>
<td>Quantum yield</td>
<td>0.0718</td>
<td>0.0667</td>
<td>0.0542</td>
<td>0.0631</td>
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<tr>
<td>Std. error</td>
<td>0.00094</td>
<td>0.0013</td>
<td>0.00096</td>
<td>0.00090</td>
</tr>
<tr>
<td>Significance</td>
<td>F = 89.4</td>
<td></td>
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<td>F = 3.1</td>
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<tr>
<td></td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
<td>P = 0.080</td>
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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 ($P_{\text{net}}$), dark respiration ($R_{\text{dark}}$), 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 $= 23$, $P < 0.0001$).
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 10°C 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.
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 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 CO₂.

If CO₂ was lowered to ambient CO₂, 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 CO₂. At 35C, growth was severely stunted, and the respiratory demands may not have been met in this warm environment. At 21C in ambient CO₂, 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂. It is well established theoretically, but often ignored experimentally, that the temperature optimum for C3 species should increase several degrees under elevated CO₂ (Harley and Tenhunen, 1991; Long, 1991). Photorespiration is reduced substantially by elevating CO₂, 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 35°C (Farquhar 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 31°C (Reddy et al., 1993), while broccoli has a optimum of only 21°C (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 ($P_{\text{gross}}$). 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 25°C) 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 (*Lactuca 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 x 0.4 x 0.9 m (L x W x H) and fully enclosed a hydroponic tub. Chamber temperature was controlled to within ±0.2C of set
point, and CO\textsubscript{2} controlled to within ±2\% of set point of 1200 \textmu\text{mol} \text{mol}^{-1}. CO\textsubscript{2} was elevated to ensure that photosynthesis would be light- rather than CO\textsubscript{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\textsubscript{2}-enriched air as that used in the canopy. CO\textsubscript{2}-gas-exchange of each of ten different whole canopies was monitored once every 10 minutes. CO\textsubscript{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\textsubscript{2} was calculated using pre-CO\textsubscript{2} minus post-CO\textsubscript{2} concentrations, and photosynthetic and respiration rates were calculated by multiplying the delta CO\textsubscript{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\textsubscript{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 \textmu\text{mol} m^{-2} s^{-1} ± 30 \textmu\text{mol} m^{-2} s^{-1} using a 16-h photoperiod to provide 35.6 mol photons per m\textsuperscript{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:

\[
\text{CQY} = \frac{P_{\text{gross}}}{\text{light absorbed}} = \frac{\text{mol C fixed}}{\text{mol photon absorbed}}
\]

\(P_{\text{gross}}\) 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 (\(P_{\text{gross}}\)) is a calculated term that incorporates both the net C fixed (\(P_{\text{net}}\)) and the C that is simultaneously being respired. \(P_{\text{gross}}\) can be calculated as:

\[
P_{\text{gross}} = P_{\text{net}} + R_d.
\]

Net photosynthesis (\(P_{\text{net}}\)) and night-time respiration (\(R_n\)) were measured directly. Since day-time respiration (\(R_d\)) can not be measured directly, \(P_{\text{gross}}\) is calculated as the sum of \(P_{\text{net}}\) 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

\[
R_n \times \frac{\text{time in light}}{\text{time in darkness}}
\]

so for a 16-h photoperiod, \(R_d = R_n \times 2\). 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:

$$\text{CUE} = \frac{P_{\text{gross}}}{\text{DCG}}$$

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

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

*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 Ca(NO₃)₂ 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 NH₄⁺ form. This is important because NH₄⁺ 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). CO₂ 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 ± 25 µmol m⁻² s⁻¹ and another at 1000 µmol m⁻² s⁻¹ ± 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 ± 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:

\[
\frac{\{(S*5) + (M*3) + (L)\} \times 100}{P} = S
\]

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 \( \mu\text{mol m}^{-2} \text{s}^{-1} \)) 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 \( \mu\text{mol m}^{-2} \text{s}^{-1} \) was observed in the initial PPF response study (Figure A-5). Yield nevertheless increased beyond 500 \( \mu\text{mol m}^{-2} \text{s}^{-1} \). 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.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Tipburn Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buttercrunch</td>
<td>Air on meristem</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>95</td>
</tr>
<tr>
<td>Waldmann’s Green</td>
<td>Air on meristem</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>60</td>
</tr>
</tbody>
</table>

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 nighttime 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.
References


Jie, H. and L. S. Kong. 1997. Growth and photosynthetic responses of three aeroponically grown lettuce cultivars (Lactuca sativa L. to different rootzone temperatures


Figure A-1. Photosynthesis and respiration rates for lettuce canopies grown in 1200 μmol mol⁻¹ CO₂ 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 CO₂ 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

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Awards —
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- NCR-101 / UK Controlled Environment Users Group meeting; September 2001
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"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.


Publications
Refereed publications —


— Non-Refereed Publications / Conference Proceedings —