# Real-time imaging of ground cover: Relationships with radiation capture, canopy photosynthesis, and daily growth rate

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**Abstract:**

Cumulative absorbed radiation is highly correlated with crop biomass and yield. Here we describe the use of a digital camera and commercial imaging software for estimating daily radiation capture, canopy photosynthesis, and relative growth rate. Digital images were used to determine percent ground cover of lettuce *(Lactuca sativa* L.) communities grown at five temperatures. Plants were grown in a steady-state, 10-chamber $CO_2$ gas exchange system, which was used to measure canopy photosynthesis and daily carbon gain. Daily measurements of percent ground cover were highly correlated with daily measurements of both absorbed radiation ($r^2 \geq 0.99$) and daily carbon gain ($r^2 \geq 0.99$). Differences among temperature treatments indicated that these relationships were influenced by leaf angle, leaf area index, and chlorophyll content. An analysis of the daily images also provided good estimates of relative growth rates, which were verified by gas exchange measurements of daily carbon gain. In a separate study we found that images taken at hourly intervals were effective for monitoring real-time growth. Our data suggest that hourly images can be used for early detection of plant stress. Applications, limitations, and potential errors are discussed.

## Introduction

We have long known that crop yield is determined by the efficiency of four component processes: 1) radiation capture, 2) quantum yield, 3) carbon use efficiency, and 4) carbon partitioning efficiency (Charles-Edwards, 1982; Penning de Vries and van Laar, 1982; Thornley, 1976). More than a half century ago, Watson (1947, 1952) showed that variation in radiation capture accounted for almost all of the variation in yield between sites in temperate regions, because the three other components are relatively constant when the crop is not severely stressed. More recently, Monteith (1977) reviewed the literature on the close correlation between radiation capture and yield. Bugbee and Monje (1992) demonstrated the close relationship between absorbed radiation and yield in an optimal environment.

Radiation capture cannot be measured directly. Early models predicted canopy light interception based on the leaf area index (LAI) and an extinction coefficient (k) that is relative to the average leaf angle following the equation (Campbell and Norman, 1998):

$$\text{Fractional light interception} = [1-e^{-(k*LAI)}] \quad [1]$$

A more direct method involves measuring photosynthetically active radiation (PAR) above and below a plant canopy with a line quantum sensor as described by Gallo and Daughtry (1986). The inherent difficulties in measuring LAI or PAR throughout a canopy and advances in radiometric techniques have led to the development of methods for remotely sensing radiation capture.

Radiometric methods rely on differences in the spectral reflectance of vegetation and soil. Vegetative indices based on reflectance in broad wavebands have provided good estimates of radiation capture, LAI, and yield in crop plants (Hatfield et al., 1984; Gallo et al., 1985). Vegetation indices have also provided good estimates of fractional ground cover (Boisard et al., 1992; Elvidge et al., 1993; White et al., 2000). More recently, spectroradiometers capable of measuring narrow band radiation have been used to monitor plant stress (Elvidge and Chen, 1995). Radiometric satellite data are now available for the evaluation of large areas, and small portable radiometers are becoming less expensive as the technology progresses.

Photographic imaging was used to evaluate ground cover and crop health as early as 1960, well before the development of radiometric methods (Blazquez et al., 1981). Early photographic methods required an observer to subjectively differentiate between soil and plant cover. Automated methods for digital image analysis were not developed until much later (Hayes and Han, 1993; van Henten and Bontsema, 1995; Beverly 1996). The disadvantages of these automated methods were that they generally required complex and expensive instrumentation and were prone to error as soil and plant color changed.

Recent advances in high-resolution digital cameras and associated image manipulation software provide enhanced methods of visual discrimination and computer thresholding that are user-friendly and inexpensive. Three recent studies have demonstrated the accuracy of digital imaging analysis for monitoring plant growth. Paruelo et al. (2000) described a method for estimating aboveground biomass in semiarid grasslands using digitized photographs and a DOS-based program they developed. Purcell (2000) described a method for measuring canopy coverage and light interception in soybean fields using a digital camera and standard imaging software. Richardson et al. (2001) described a digital method for quantifying turfgrass cover following a modified version of Purcell (2000).

Here we describe how daily digital imaging of ground cover can be used to predict radiation capture, canopy photosynthesis and relative growth rate in lettuce grown in a controlled environment. We also evaluate the potential for using hourly digital imaging to predict short-term stress.

## Materials and Methods

### Cultural conditions

Lettuce (*Lactuca sativa* L. 'Grand Rapids') seedlings were germinated in blotter paper and transplanted four days after imbibition. Seedlings were arranged uniformly at a density of 106 plants $m^{-2}$. Plants were grown hydroponically. The $CO_2$ concentration was maintained at 1200 umol $mol^{-1}$ $CO_2$, an optimal level for growth in controlled environments (Bugbee et al., 1994).

Five day/night temperature set points were used: 21/18, 26/21, 29/24 , 32/27, and 35/30$^oC$, with two replicate chambers per treatment. Root-zone solution temperature was controlled to the daily average temperature. Air temperature control was within ±0.2$^oC$ of the set point and root temperatures were within ±0.5$^oC$ of set point. Relative humidity was maintained between 60 and 80%. High-pressure sodium lamps (HPS) placed above a water barrier provided a photosynthetic photon flux (PPF) of 600 μmol $m^{-2}$ $s^{-1}$ for a 16-h photoperiod. Reflective mylar sheeting wrapped around each chamber was used to minimize side lighting.

## Ground cover

A two-megapixel digital camera was placed on top of a chamber, 68 cm above the plant base, to take daily images of the lettuce until canopy closure. Images were processed in Adobe Photoshop (Adobe Systems Inc., 2001) using the “magic wand” thresholding tool and “select similar” command to separate plants from the background. Percent ground cover was determined by using the “histogram” function. This function shows the number of pixels in a given selection thus making it possible to determine the percent ground cover without knowing the actual dimensions of a plot. Ground cover estimates were corrected for error in apparent size using the distance formula

$$\text{Apparent size} \propto d_1^2/d_2^2 \quad [2]$$

where $d_1$ is the original average distance from the camera to the canopy, and $d_2$ is the new average distance to the canopy (see discussion). The correction factor reached a maximum of 11 % when the average canopy height was 3.5 cm. Corrected percent ground cover was defined as :

$$\text{Percent cover} = (\text{plant pixel number} \times d_2^2/d_1^2)/\text{plot area pixel number} \times 100 \quad [3]$$

Hourly images were also made on a single lettuce plant over a three-day period to evaluate the potential for monitoring short-term plant growth. A camera with an automated timer capable of taking hourly images was used (Nikon, Coolpix 995). This was the only digital camera with an automated timer available at the time of this writing.

## Radiation absorption

Radiation capture was determined from the ratio of absorbed ($PPF_{abs}$) to incident PPF ($PPF_O$) as defined by Gallo and Daughtry (1986):

$$PPF_{abs} = PPF_O - PPF_{RO} - PPF_T + PPF_{RT} \quad [4]$$

where $PPF_{RO}$ is the reflected incident, $PPF_T$ is the transmitted, and $PPF_{RT}$ is the transmitted PPF reflected by the media. Four measurements per chamber of each component were measured daily with a 35-cm long line quantum sensor (Apogee Instruments Inc., Logan, UT) calibrated for HPS lamps.

**Photosynthesis and daily carbon gain**

Photosynthesis was measured in a 10-chamber, semi-continuous gas exchange system, which has been described previously (van Iersel and Bugbee, 2000). Each chamber is 0.5 x 0.4 x 0.9 m (L x W x H). Separate hydroponic systems fit entirely inside each chamber (Fig. 1). Hydroponic solution was bubbled with the same air as that used in the shoot. The pH of the hydroponic solution was maintained between 4 and 5 in order to minimize $CO_2$ dissolved in solution (Monje and Bugbee, 1998). A mass-flow controller maintained the $CO_2$ to within 2% of set point. $CO_2$ measurements were made using two infrared gas analyzers (LI-COR model 6251, Lincoln, NE), one in absolute mode and one in differential mode. A pre/post-chamber $\Delta CO_2$ was measured with a differential analyzer. Photosynthesis and respiration rates were calculated by multiplying the $\Delta CO_2$ by the flow rate as previously described for 'open' gas-exchange systems (Bugbee, 1992).

Daily carbon gain was calculated as:

$$\text{Daily carbon gain} = \text{Pnet} - \text{Rn} \quad [5]$$

Where Pnet is the integrated net daily photosynthesis (mol C $m^{-2}$ $d^{-1}$) and Rn is the integrated nighttime respiration (mol C $m^{-2}$ $night^{-1}$). A summation of the daily carbon gain values provides a measurement of the total moles of carbon in the plant community at harvest. This value, multiplied by the molar mass of carbon (12), divided by the dry

**Fig. 1. Photograph of the semi-continuous ten-chamber gas exchange system fitted with independent hydroponic tubs.**

biomass, predicts the percent carbon in the tissue. Gas exchange measurements predicted 39 % carbon in the final biomass. Tissue analysis, by combustion, resulted in 38.6% carbon, demonstrating that gas exchange had an accuracy of 99%. This accuracy is similar to values in previous studies (Monje and Bugbee, 1998).

**Data analysis**

Comparisons between percent ground cover, PAR absorption and daily carbon gain were similar in replicate treatments, so data for only a single, randomly selected, replicate are shown to simplify interpretation.

## Results and Discussion

### Radiation capture

Ground cover was a good predictor of canopy PAR absorption ($r^2 \geq 0.99$) at each temperature, but the ratio of radiation absorption to ground cover tended to increase with increasing temperature (Fig. 2). Plants in the warmer treatments were more erectile, had more leaves per unit area, and were greener than plants in cooler treatment. These changes resulted in lower levels of reflected and transmitted radiation per unit ground cover (Fig. 3). This trend dropped off in the 33°C treatment, which clearly experienced high temperature stress.

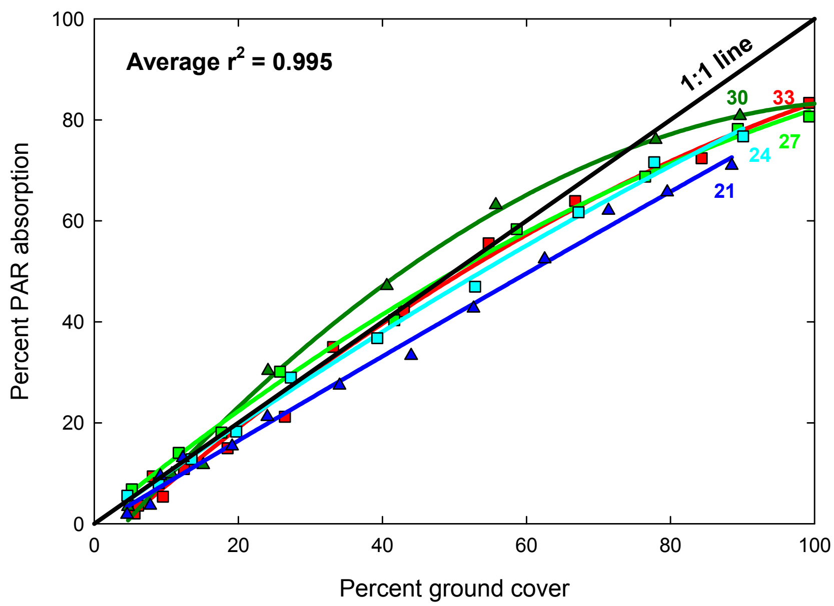


**Fig. 2. The relationship between percent ground cover measured using digital imagery and percent PAR absorption measured using a light bar for lettuce grown at five temperatures from 21 to 33ºC. The average day and night temperature is shown for each treatment for all the figures.**

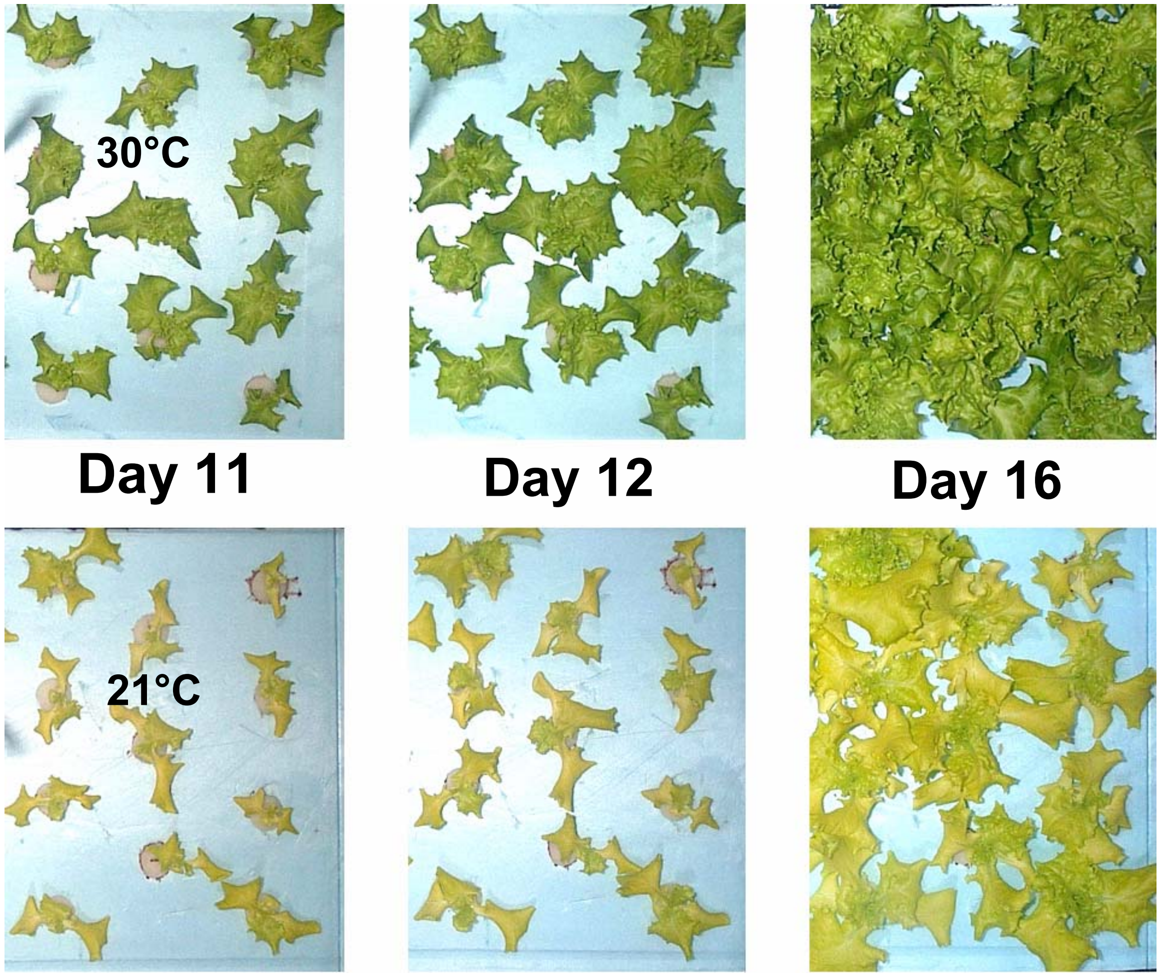


**Fig. 3. Successive overhead pictures of the 21°C (bottom) and 30° C (top) canopies showing the differences in color and growth between these treatments. Also note the spatial variability in plant cover that contributes to error when making measurements of PAR absorbtion with a light bar.**

The relationship between ground cover and PAR absorption was nearly 1:1 but was generally nonlinear and thus varied over time. This ratio especially deviated from 1:1 at canopy closure. This was expected since a canopy can reach 100% ground cover, but will never reach 100 % PAR absorption.

## Photosynthesis and daily carbon gain

Ground cover was highly correlated with daily carbon gain ($r^2 \geq 0.99$) at each temperature (Fig. 4). Consistent with trends observed with PAR absorption, the ratio of carbon gain to ground cover increased with increasing temperature. This was expected since warmer treatments had more erectile growth, a higher leaf area index (LAI), and greener leaves than cooler treatments. PAR absorption is more directly related to daily carbon gain than ground cover, and the relationship was thus more linear (Fig. 5). Although the relationship between ground cover and carbon gain was non-linear, predicted values fell within 10% of actual values for all but the first day of measurements.

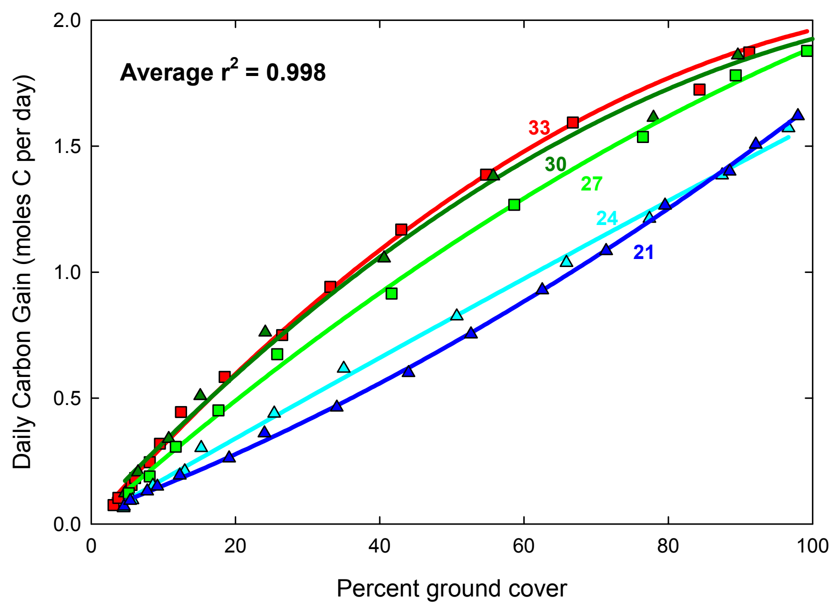


**Fig. 4. The relationship between percent ground cover measured using digital imagery and daily carbon gain measured by gas exchange for lettuce grown at five temperatures from 21 to 33ºC.**

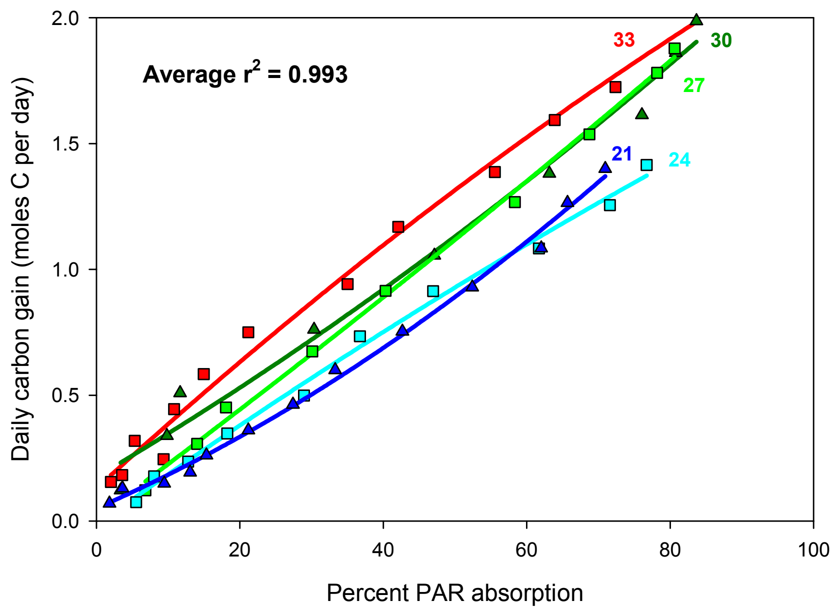


**Fig. 5. The relationship between percent PAR absorption and daily carbon gain for lettuce grown at five temperatures from 21 to 33ºC.**

Based on daily carbon gain the 30°C treatment was optimal and the two temperature extremes (21 and 33° C) had the slowest growth rates (Fig. 6). Percent cover predicted similar trends but the relative differences between treatments were not accurately reflected (Fig. 7). The most obvious error was in the difference between the 21° and 33°C treatments, again showing how differences in leaf angle, LAI, and chlorophyll content influenced the relationship between percent ground cover and carbon gain.

While daily measurements of percent cover would require calibration to be used for measuring absolute growth rates, this method can be used directly for monitoring *relative* growth rates. For example, the relative growth rate (RGR) of a crop is defined

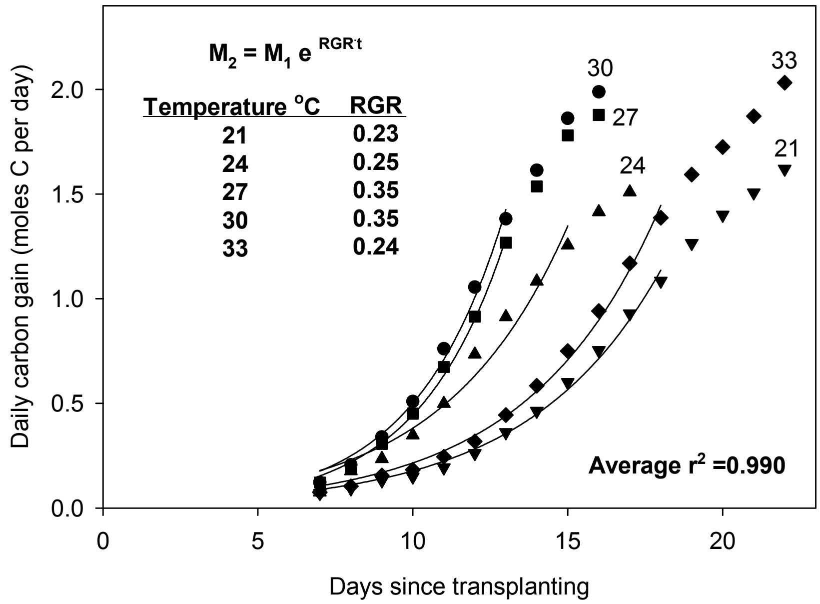


**Fig. 6. Growth curves showing the daily carbon gain of the five lettuce communities up to canopy closure. Data were fitted to an equation derived from the relative growth rate equation where $M_2$ = final daily carbon gain, $M_1$ = initial daily carbon gain, RGR= relative growth rate, and t = time.**

by:

$$RGR = \ln (M_2/M_1)/\Delta t \quad [6]$$

where $M_1$ is the initial dry mass, $M_2$ is the final dry mass, and $\Delta t$ is the change in time in days. Solving for $M_2$, this equation can be rewritten as:

$$M_2 = M_1 e^{RGR \cdot \Delta t} \quad [7]$$

This equation, in the form of $y = ae^{bx}$ (a standard curve fitting equation in graphing software), was used to curve fit the data in Figure 6 by substituting daily carbon gain for dry mass. The curve fit was limited to early growth since the RGR declines as the canopy approaches 100 % ground cover, as shown by the unfitted points in Figure 6. Curve fits using percent PAR absorbed and percent ground cover in place of dry mass resulted in similar estimates of RGR for all five temperature treatments (Figs. 7 & 8).

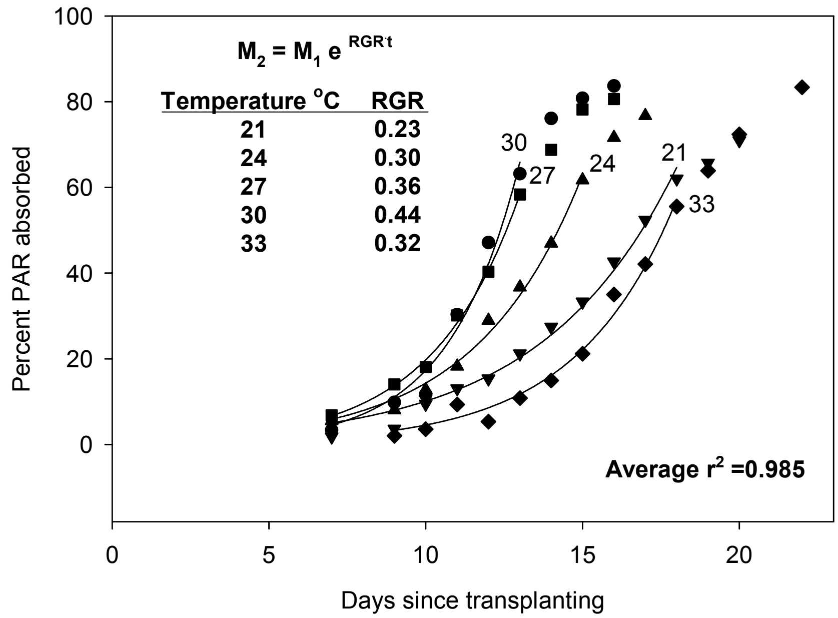


**Fig. 7. Growth curves showing the percent PAR absorbtion of five lettuce communities over time. Data were fitted to an equation derived from the relative growth rate equation where $M_2$ = final percent PAR absorbed, $M_1$ = initial percent PAR absorbed, RGR= relative growth rate, and t = time.**

RGR was overestimated by both PAR absorption and ground cover in all but the 21ºC treatment, but the relative ranking of the treatments was accurately determined. This suggests that the photosynthetic efficiency (quantum yield) was lower in the higher temperature treatments and that leaf expansion increased more rapidly than daily carbon gain.

This type of analysis is analogous to a nondestructive method for measuring radiation use efficiency (RUE; g biomass per MJ of intercepted radiation) since digital imagery is highly correlated to both carbon gain and absorbed radiation. Thus daily measurements of ground cover can be very useful for monitoring the relative growth and RUE of a crop.

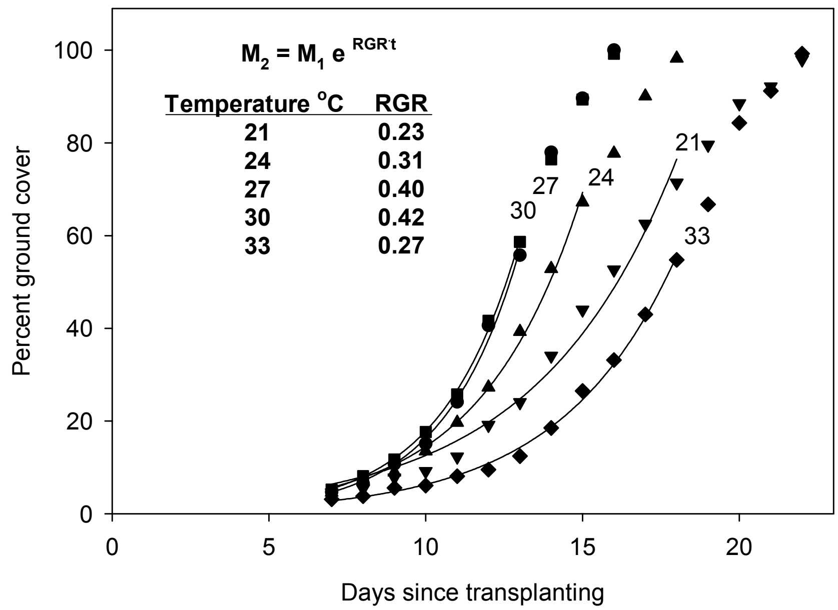


**Fig. 8. Growth curves showing the percent ground cover of five lettuce communities over time. Data were fitted to an equation derived from the relative growth rate equation where $M_2$ = final percent cover, $M_1$ = initial percent cover, RGR= relative growth rate, and t = time.**

### Hourly imaging

Preliminary data on a single plant indicated that we could monitor hourly growth using an automated digital camera. The increase in size of a single lettuce plant was measured by overhead images taken hourly during the light period for 7 days (Fig. 9). The relative growth rate of the plant was calculated from the increase in size by substituting the megapixel data for dry mass in equation 7. An RGR of 0.34 grams per gram per day is typical for a young plant in favorable environmental conditions. This analysis of hourly measurements may prove to be a useful tool for the early detection of plant stresses that influence rates leaf expansion such as drought or flooding stress. Additional testing is in progress to improve on this method and to determine how to minimize sources of error.

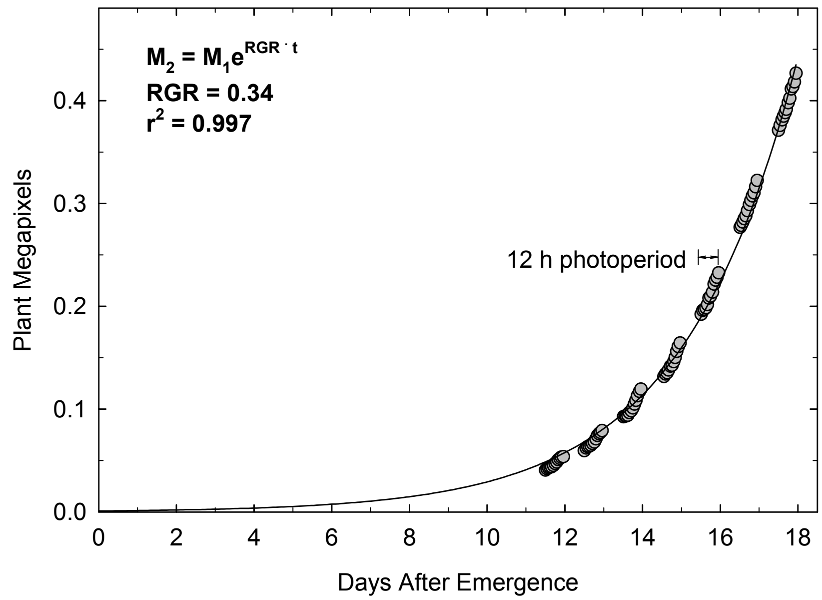


**Fig. 9. The increase in size of a single lettuce plant as measured by hourly overhead images taken during a 12-h photoperiod for 7 days. Data were fitted to an equation derived from the relative growth rate equation where $M_2$ = final plant megapixels, $M_1$ = initial plant megapixels, RGR= relative growth rate, and t = time.**

**Errors in estimating radiation capture and percent ground cover**

The biggest error with using a light bar is in getting an accurate measurement of the transmitted radiation. Transmitted light is highly spatially variable prior to canopy closure thus requiring numerous measurements to get an accurate result. This is even a greater problem when plants grow non-uniformly within a plot as was observed in the highest temperature treatment (33$^{o}$C). Radiation capture is inherently underestimated because the light bar cannot be place directly under each plant. The relationship between ground cover and radiation capture was not quite 1:1 but it was close. The greater deviation from a 1:1 relationship observed between PAR absorption and ground cover when the plants were small is consistent with an underestimation of radiation capture using a light bar (Fig. 1). In contrast, a digital image effectively integrates all the spatial variability into a single measurement and includes the portion of a plant that a light bar cannot get under.

Digital imaging of percent ground cover tends to overestimate radiation capture because individual leaves and plants never absorb 100% of the incident light. This is especially evident at canopy closure (100% ground cover) when canopy PAR absorption remains below 100%. Canopies will have a higher PAR absorption per unit ground cover as leaf angle, LAI, chlorophyll content, and specific leaf mass (SLM), increase, and thus have a ground cover to PAR absorption ratio closer to 1:1. Of these factors, only increased leaf angle can cause digital imaging to *under*estimate PAR absorption. This is most evident with a vertical leaf which will appear to have virtually 0% ground cover but still absorbs radiation.

Light bar measurements made in the field must be made near solar noon since diurnal variation in sun angle will significantly influence this measurement. Purcell (2000) demonstrated that digital imaging of percent ground cover was relatively insensitive to diurnal variation. Purcell (2000) suggested that a camera angle similar to the solar elevation angle helps to minimize error associated with partial shading of canopy cover. The definition between canopy and background will be optimized in diffuse light situations commonly found in controlled environments. We found that pictures taken perpendicular to the canopy worked well in the diffuse light of a growth chamber. Use of a flash may help to improve an image but is not required to get adequate pictures for evaluating ground cover.

A potential ground cover measurement error in near-remote imaging (<5 m) involves vertical plant growth. The apparent size of an object increases as it approaches the camera proportional to the square of the distance from the camera. Plants growing toward a set camera, increase in apparent size and cause an overestimation of ground cover. This problem is negligible if plant growth is small in comparison to the distance from the camera to the plant, but can result in large errors if the camera is close to the plants. For example, a 1% decrease in the distance between a canopy and the camera results in a 2% error but a 10% change would result in a 23% error. This error is particularly difficult to correct for because overhead images may show leaves at all heights from the base to the top of the canopy. Changes in apparent size as plants grew were accounted for in our tests by measuring mean canopy height and removing the effect of apparent size based on the distance formula (equation 2).

## Conclusions

Measurements of PAR absorption are a more fundamental indicator of plant growth than percent ground cover. However, in practice, digital imaging of ground cover is simpler and less prone to error since it requires far fewer measurements, which are less sensitive to spatial and temporal variation. Percent ground cover is highly correlated with radiation capture and photosynthesis but the relationships are dependent on canopy morphology, color, and stage of canopy development. Radiation capture was underestimated by daily measurements of percent cover in more erectophile canopies and overestimated in more horizontal and lighter green canopies. Percent cover inherently overestimates radiation capture at canopy closure and is thus most useful for monitoring early stages of growth. Daily imaging of ground cover provided good estimates of the RGR of lettuce prior to canopy closure and this relationship was less sensitive to differences in canopy morphology or color. The ability to measure the RGR on an hourly basis suggests digital imaging could be developed into a powerful tool for monitoring short-term stress responses in plants.

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