Photosynthetic and Transpiration Responses to Light, CO$_2$, Temperature, and Leaf Senescence in Garlic: Analysis and Modeling

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ABSTRACT. Characterization of leaf physiology is an important step for understanding the ecophysiology of a crop as well as for developing a process-based crop simulation model. We determined photosynthetic and transpiration responses to photosynthetic photon flux (PPF), carbon dioxide concentrations, and temperature, and parameterized a coupled leaf gas-exchange model for hardneck garlic (Allium sativum). The parameterized model performed with high accuracy and precision in predicting photosynthetic responses ($r^2 = 0.95$, bias = 1.7 $\mu$mol m$^{-2}$s$^{-1}$) when tested against independent data that were not used for model calibration. The model performance for transpiration rates was less satisfactory ($r^2 = 0.49$, bias = $–0.14$ mmol m$^{-2}$s$^{-1}$, RMSE = 0.94 mmol m$^{-2}$s$^{-1}$). In addition, we characterized the relationships among chlorophyll meter readings, leaf photosynthetic capacity ($A_{\text{max}}$), and leaf nitrogen content in garlic leaves. The chlorophyll meter readings were a reasonable indicator of both $A_{\text{max}}$ ($r^2 = 0.61$) and leaf nitrogen (N) status ($r^2 = 0.51$) for garlic leaves we studied. The garlic leaf gas-exchange model developed in this study can serve as a key component in ecophysiological crop models for garlic. Similarly, the quantitative relationship identified between chlorophyll meter readings and $A_{\text{max}}$ in this study can provide useful information for non-destructively assessing leaf photosynthetic capacity in garlic.

With a long history of cultivation, garlic is an important food crop that has been incorporated into cuisines around the world. As of 2010, the crop was cultivated on $\approx$1.3 million hectares worldwide with total production reaching more than 22 million megagrams (Food and Agriculture Organization of the United Nations, 2010). In addition, garlic has been used medicinally for millennia (Rivlin, 2001) as a health supplement (Stevinson et al., 2000) and at times a panacea (Kik et al., 2001). There exists a rich literature detailing garlic botany and horticulture (Engeland, 1994; Kamenetsky, 2007), especially regarding nutrient (Bertoni et al., 1992; Buwalda, 1986b) and water inputs (Villalobos et al., 2004). Previous studies have made valuable contributions in modeling canopy responses using radiation use efficiency and water use efficiency in garlic (Rizzalli et al., 2002; Villalobos et al., 2004). Building on these efforts, a mechanistic crop simulation model that integrates the current knowledge of physiology and ecology of this important crop will improve our ability to enhance garlic yield and quality for health benefits, optimize crop management decisions, and develop adaptation strategies to reduce climate change impacts and vulnerability.

Crop models are essential tools for assessing climate impacts on crops, assisting crop breeding and management decisions, forecasting crop yield for policy and economic decisions, and developing adaptive cropping solutions in a changing climate as recently illustrated by Chen et al. (2011). Because leaves are a fundamental unit for carbon uptake and water use, a leaf physiology module is a critical component that, when built into a full crop simulation model, can be scaled to the canopy level. Leaf physiology models simulating photosynthesis and transpiration are at the core of process-based crop models for accurate predictions of crop biomass accumulation, allocation, and yield formation (Kim and Lieth, 2003; Lizaso et al., 2005). Coupled leaf gas-exchange models of photosynthesis and transpiration are a useful modeling approach in that they mechanistically interface carbon, water, and energy balances governing physical, biochemical, and physiological processes involved in leaf gas exchange (Kim and Lieth, 2003). These models have become essential tools for assessing climate impacts on forest and other ecosystems (e.g., Thornton et al., 2002) and for studying biosphere–atmosphere interactions (Krinner et al., 2005). However, most crop simulation models have yet to adopt the coupled model approach with a few exceptions (e.g., Da Silva et al., 2011; Kim et al., 2012). To build a leaf physiology model, it is critical to characterize photosynthetic responses to a wide range of key environmental factors including PPF, CO$_2$, and temperature. However, limited information is available on garlic leaf physiology in the literature.

Garlic has moderate to high N demand with an estimate of 80 to 170 kg·ha$^{-1}$ in California (Rosen and Tong, 2001) or 120 kg·ha$^{-1}$ in New Zealand as an optimal level (Buwalda, 1986a). A nondestructive optical method using a chlorophyll meter (e.g., SPAD 502; Konica Minolta, Ramsey, NJ) is useful in determining crop nutrient status (e.g., N) without destructively harvesting plants. For example, chlorophyll meters have been widely used for assessing plant N status in various agronomic and horticultural crops such as corn [Zea mays (Yang et al., 2012)], rice [Oryza sativa (Peng et al., 1999)], apple [Malus pumila (Neilson et al., 1995)], and potato [Solanum tuberosum (Uddling et al., 2007)]. In many crops, chlorophyll meter readings are closely related to leaf N status but this relationship
can also be shifted by other macro- and micronutrients such as phosphorus and boron (Peng et al., 1999; Sotiropoulos et al., 2006). Chlorophyll meter readings may be used as a surrogate for leaf photosynthetic capacity if these readings can reflect leaf senescence and nutritional status that determine the functioning of photosynthetic apparatus. A method that links chlorophyll meter readings with leaf photosynthetic capacity may provide a practical yet mechanistic method to determine crop photosynthesis as a surrogate of production potential.

In this study, we characterized photosynthetic and transpiration responses of garlic leaves and applied the results to parameterize a coupled leaf gas-exchange model developed by Kim and Lieth (2003). This model was developed for cut-flower rose (*Rosa ×hybrida*), but has been extended to and adopted for other crop models such as potato (Fleisher et al., 2010), peach (*Prunus persica* [Da Silva et al., 2011]), rose (Buck-Sorlin et al., 2011), cucumber (*Cucumis sativus* [Wiechers et al., 2007]), and *Scavola aemula* (Kim et al., 2007). Specifically, the aims of our study were to 1) evaluate photosynthetic responses of garlic leaves to light, temperature, and CO<sub>2</sub>; 2) parameterize a coupled gas-exchange model for garlic leaves; and 3) test the model performance against an independent data set not used for parameterization. We also tested if and how chlorophyll meter readings are related to photosynthetic capacity and leaf N content during senescence in garlic leaves. This study fills a gap in our knowledge of garlic ecophysiology that is fundamental for developing a mechanistic, process-based crop model.

Materials and Methods

**PLANT MATERIALS.** Ninety seed cloves of an Asiatic hardneck garlic (cv. Japanese Mountain) purchased from Filaree Garlic Farm (Okanogan, WA) were planted at a density of 18.5 plants/m<sup>2</sup> in three raised beds (1.8 m long × 1.2 m wide × 0.4 m high) at the Center for Urban Horticulture, University of Washington

| Symbol | Description | Units | Estimate ± se<sup>a</sup> |
|--------|-------------|-------|---------------------------|
| A      | Leaf net CO<sub>2</sub> assimilation rate. A is determined by the minimum of <em>A</em><sub>c</sub>, <em>A</em><sub>j</sub>, and <em>A</em><sub>p</sub> minus dark respiration rate (<em>R</em><sub>d</sub>) [i.e., <em>A</em> = min(<em>A</em><sub>c</sub>, <em>A</em><sub>j</sub>, <em>A</em><sub>p</sub>) – <em>R</em><sub>d</sub>] | μmol m<sup>-2</sup> s<sup>-1</sup> | |
| <em>A</em><sub>c</sub> | Rubisco-limited CO<sub>2</sub> assimilation rate | μmol m<sup>-2</sup> s<sup>-1</sup> | |
| <em>A</em><sub>j</sub> | Electron transport-limited CO<sub>2</sub> assimilation rate | μmol m<sup>-2</sup> s<sup>-1</sup> | |
| <em>A</em><sub>max</sub> | Light saturated <em>A</em> at ambient <em>C</em><sub>a</sub> (≈400 μmol·mol<sup>-1</sup>) | μmol m<sup>-2</sup> s<sup>-1</sup> | |
| <em>A</em><sub>p</sub> | Triose phosphate utilization-limited CO<sub>2</sub> assimilation rate | μmol m<sup>-2</sup> s<sup>-1</sup> | |
| <em>b</em> | Residual <em>g</em><sub>S</sub> to water vapor. Also known as <em>g</em><sub>0</sub> | mol m<sup>-2</sup> s<sup>-1</sup> | 0.096<sup>b</sup> |
| <em>C</em><sub>a</sub> | Atmospheric CO<sub>2</sub> concentration | μmol·mol<sup>-1</sup> | |
| <em>C</em><sub>i</sub> | CO<sub>2</sub> concentration in intercellular air spaces | μmol·mol<sup>-1</sup> | |
| <em>E</em> | Leaf transpiration rate | mmol·m<sup>-2</sup> s<sup>-1</sup> | |
| <em>g</em><sub>S</sub> | Stomatal conductance to water vapor | mol m<sup>-2</sup> s<sup>-1</sup> | |
| <em>K</em><sub>c</sub> | Michaelis-Menten constant of Rubisco for CO<sub>2</sub> | μmol·mol<sup>-1</sup> | |
| <em>K</em><sub>o</sub> | Michaelis-Menten constant of Rubisco for O<sub>2</sub> | μmol·mol<sup>-1</sup> | 404.9<sup>c</sup> |
| <em>J</em> | Rate of electron transport | μmol m<sup>-2</sup> s<sup>-1</sup> | 278.4<sup>c</sup> |
| <em>J</em><sub>max25</sub> | Potential rate of electron transport at 25 °C | μmol m<sup>-2</sup> s<sup>-1</sup> | 169.0 ± 10.30 |
| <em>J</em><sub>max</sub> | Potential rate of electron transport at 25 °C | μmol m<sup>-2</sup> s<sup>-1</sup> | |
| <em>m</em> | Empirical coefficient for the sensitivity of <em>g</em><sub>5</sub> to <em>A</em>, CO<sub>2</sub>, and relative humidity. Also known as <em>g</em><sub>5</sub> | — | 6.82 ± 0.36 |
| <em>P</em><sub>u25</sub> | Rate of triose phosphate utilization at 25 °C | μmol·m<sup>-2</sup> s<sup>-1</sup> | 16.03 ± 1.29 |
| <em>R</em><sub>d</sub> | Dark respiration rate. Same values used for day respiration rate. | μmol·m<sup>-2</sup> s<sup>-1</sup> | |
| <em>R</em><sub>d25</sub> | <em>R</em><sub>d</sub> at 25 °C | μmol·m<sup>-2</sup> s<sup>-1</sup> | 1.08 ± 0.13 |
| <em>V</em><sub><em>c</em>max25</sub> | Photosynthetic Rubisco capacity at 25 °C | μmol·m<sup>-2</sup> s<sup>-1</sup> | 108.4 ± 5.19 |
| <em>V</em><sub><em>c</em>max</sub> | Photosynthetic Rubisco capacity | μmol·m<sup>-2</sup> s<sup>-1</sup> | |
| <em>Γ</em><sup>*</sup> | CO<sub>2</sub> compensation point in the absence of day respiration | μmol·mol<sup>-1</sup> | 42.75<sup>−</sup> |

Temperature dependence parameters

| Symbol | Description | Units | Estimate ± se<sup>a</sup> |
|--------|-------------|-------|---------------------------|
| <em>E</em><sub>a</sub> | Activation energy determining exponential rate increase before the peak for: | kJ·mol<sup>-1</sup> | 24.0 ± 2.1 |
| <em>J</em><sub>max</sub> | | | |
| <em>J</em><sub>max</sub> | | | |
| <em>K</em><sub>c</sub> | | kJ·mol<sup>-1</sup> | 79.43<sup>c</sup> |
| <em>K</em><sub>o</sub> | | kJ·mol<sup>-1</sup> | 36.38<sup>c</sup> |
| <em>P</em><sub>n</sub> | | kJ·mol<sup>-1</sup> | 47.1<sup>c</sup> |
| <em>R</em><sub>d</sub> | | kJ·mol<sup>-1</sup> | 46.39<sup>c</sup> |
| <em>V</em><sub><em>c</em>max</sub> | | kJ·mol<sup>-1</sup> | 52.16 ± 7.1 |
| <em>Γ</em><sup>*</sup> | | kJ·mol<sup>-1</sup> | 37.13<sup>c</sup> |
| <em>H</em><sub>j</sub> | | kJ·mol<sup>-1</sup> | 200<sup>c</sup> |
| <em>S</em><sub>j</sub> | | kJ·mol<sup>-1</sup> | 616.4<sup>c</sup> |

<sup>a</sup>Other model parameters not specified here were used as in Kim and Lieth (2003).

<sup>b</sup>All parameters were determined in this study unless noted otherwise.

<sup>c</sup>Kim and Lieth (2003).

<sup>d</sup>Bernacchi et al. (2001).

<sup>e</sup>Medlyn et al. (2002).
(Seattle) on 12 Dec. 2011. Two beds (Plots 1 and 2) were located at the mouth of Union Bay Natural Area and the third bed (Plot 3) was located in the nursery area of the Center for Urban Horticulture; Plot 3 was 200 m away from Plots 1 and 2. Commercially available topsoil (three-way soil mix composed of sandy soil, composted sawdust, and manure; Sky Nursery, Shoreline, WA) was used to fill the beds. All beds were hand-watered as needed during dry periods beginning May 2012 until harvest on 13 July. All plots were fertilized with a modified Hoagland solution at emergence on 2 Feb. and twice during growth on 30 Apr. and 9 May. A full description of the nutrient solution is detailed in Kinmonth-Schultz and Kim (2011). In addition, controlled-release fertilizer [CRF (15N–3.5P–10.0K, Osmocote Plus Multi-Purpose Plant Food; Scotts, Maryville, OH)] was applied on 30 Apr.; the fertilizer supplied N as 8% NH₄⁺ and 7% NO₃⁻. Total N supplied during the experiment amounts to 202 kg ha⁻¹ (36 kg in Hoagland solution and 166 kg as CRF); this fertilization rate is within the optimal range suggested in the literature (i.e., 60 to 240 kg ha⁻¹) (Kamenetsky, 2007). Scapes were not removed from the plants used for gas-exchange measurements to avoid physiological disruptions. For all other plants, scapes were removed on 16 or 17 May 2012.

**Leaf gas-exchange measurements.** A portable photosynthesis system with a leaf chamber fluorometer (LI-6400-40; LI-COR, Lincoln, NE) was used to measure the rate of net CO₂ assimilation (A), stomatal conductance (gs), and transpiration (E). Gas-exchange measurements were made on fully expanded leaves from 15 randomly selected plants from the three plots during the bulbing stage in late May and June. Photosynthetic-CO₂ response (A-Cᵢ) curves were logged at nine reference CO₂ levels: 0, 50, 100, 200, 300, 400, 700, 1000, and 1500 μmol·mol⁻¹ at four different leaf temperatures: 17, 24, 35, and 39 °C with a minimum wait time of 3 min before each logging. Ambient air temperatures during the periods of data collection ranged between 13.0 and 27.8 °C. The PPF was maintained at 1500 μmol·m⁻²·s⁻¹ and relative humidity greater than 40%. Photosynthetic light responses (A-Q) were also measured at 11 different PPF levels between 0 and 2000 μmol·m⁻²·s⁻¹ with reference CO₂ set to 400 μmol·mol⁻¹ and block temperature set to 25 °C. We monitored leaf greenness as a surrogate of leaf nutrition and senescence status using a chlorophyll meter (SPAD 502). Gas-exchange data from fully expanded young leaves that covered the cuvette leaf area (2 cm²) with little or no sign of senescence (chlorophyll meter readings: 46 to 66 SPAD units) were used for parameterization and testing of a coupled leaf physiology model for garlic.

The gas-exchange measurements used for model calibration were made on 20 leaves from eight plants (Plots 1 and 2) and on 12 leaves from seven plants (Plot 3) for model testing.

**Chlorophyll meter readings and leaf nitrogen content during senescence.** We evaluated how chlorophyll meter readings are related to photosynthetic capacity represented by Amax and leaf N content over the course of leaf senescence. Two to three fully elongated upper leaves on each of five to seven plants per plot were selected for chlorophyll meter measurements during the growing and senescing periods. A minimum of five chlorophyll meter readings was averaged for each leaf. Sampling of leaves for chlorophyll meter readings and leaf N determination included fully green mature leaves, senescing leaves, and senesced leaves to ensure that a wide range of chlorophyll meter readings was included in the data. An additional set of leaf gas-exchange data was collected on selected leaves with chlorophyll meter readings during early to late senescence to determine Amax under saturating light (1500 μmol·m⁻²·s⁻¹), ambient CO₂ (≈400 μmol·mol⁻¹), and optimal leaf temperature (≈25 °C). Note that data from senesced leaves were not included for leaf gas-exchange model calibration and testing described in the following section. The leaves used for chlorophyll meter readings were harvested for determination of leaf area, dry weight, specific leaf area (leaf

![Image](210x119 to 548x447)

Fig. 1. Leaf gas-exchange model parameterization for garlic leaves. Measured and predicted net CO₂ assimilation rates (A) for calibration data. (A) Light response at leaf temperature ≈24 °C; (B) A-Cᵢ (internal CO₂) response at leaf temperature ≈24 °C; (C) temperature responses at low (square), ambient (circle), and high (triangle) (CO₂); and (D) predicted A vs. observed A of all data used for model calibration. Dashed line represents 1:1 relationship and solid line the regression. All symbols represent mean ±SE of observations (n = 3 to 5). Solid lines represent model predictions. RMSE is root mean square error between the predicted (Y) and the observed (X).
area/leaf dry weight), and leaf N concentration (w/w) using a CHN Analyzer (Model 2400; PerkinElmer, Waltham, MA). Leaf N concentration was converted to leaf N content per leaf area (grams per square meter) using specific leaf area (square meters per gram). These data were used to relate chlorophyll meter readings with $A_{\text{max}}$ and leaf N content of mature and senescing leaves using linear regression.

**Leaf gas-exchange model parameterization and testing.** The coupled leaf gas-exchange model developed for rose leaves (Kim and Lieth, 2003) was parameterized for fully expanded young garlic leaves (i.e., non-senescing) without apparent nutrient deficiency (SPAD units greater than 46) grown in Plots 1 and 2. Leaf gas-exchange data from Plot 3 were not included in parameterization but used for testing model performance as independent data. The model by Kim and Lieth (2003) couples the models of photosynthesis (Farquhar et al., 1980), stomatal conductance (Ball et al., 1987), and leaf energy balance. A stepwise calibration for different parameters was done as detailed in Kim and Lieth (2003) and Kim et al. (2007). A total of eight parameters were calibrated for garlic leaves: Rubisco capacity at 25 °C ($V_{\text{cm25}}$), maximum electron transport rate at 25 °C ($J_{\text{m25}}$), rate of triose phosphate utilization at 25 °C ($P_{n25}$), dark respiration rate at 25 °C ($R_{d25}$), stomatal sensitivity ($m$ or $g_{s}$), activation energy ($E_{a}$) for $V_{\text{cm}}$ and $J_{\text{m}}$, and entropy factor ($S_{j}$) for $J_{\text{m}}$. Calibrations and performance testing were done using SAS NLIN (Version 9.3; SAS Institute, Cary, NC) and their estimates are listed in Table 1. Model performance was evaluated based on the coefficient of determination ($r^2$), bias, and RMSE (Kim et al., 2012). All other parameter values were used as in the literature (Bernacchi et al., 2001; Kim and Lieth, 2003; Medlyn et al., 2002) (see Table 1).

**Results**

**Photosynthetic response to photosynthetic photon flux, CO₂, and temperature.** Young fully elongated garlic leaves tested in this study exhibited active photosynthetic and transpiration rates for a C₃ plant. The $A_{\text{max}}$ at saturating light ($PPF = 2000 \mu mol\cdot m^{-2}\cdot s^{-1}$) in ambient CO₂ ($\approx$400 ppm) and air temperature near 25 °C was $23.9 \pm 0.8 \mu mol\cdot m^{-2}\cdot s^{-1}$ (Fig. 1A). The transpiration rate was $4.67 \pm 0.27 \mu mol\cdot m^{-2}\cdot s^{-1}$, $g_{s}$ was $0.462 \pm 0.043 \mu mol\cdot m^{-2}\cdot s^{-1}$, and the $C_{i}/C_{o}$ ratio was $0.75 \pm 0.02$ for the same conditions (data not shown). The $A-C_{i}$ response curves revealed that $A$ reached above 30 $\mu mol\cdot m^{-2}\cdot s^{-1}$ with saturating CO₂ in moderate leaf temperatures ($\approx$25 °C); it increased further to $\approx$40 $\mu mol\cdot m^{-2}\cdot s^{-1}$ when leaf temperature was raised to near 38 °C in saturating CO₂ (1500 $\mu mol\cdot mol^{-1}$) (Fig. 1B–C). Similar to other C₃ plants, optimal leaf temperatures for garlic photosynthesis rose as CO₂ increased. That is, the maximum $A$ values were observed between 15 and 25 °C in ambient CO₂ ($\approx$400 $\mu mol\cdot mol^{-1}$) but shifted to between 35 and 40 °C when CO₂ was elevated to 1500 $\mu mol\cdot mol^{-1}$ (Fig. 1C). Bulb yield at harvest from plants with scape removed was 43.3 g of fresh weight per plant.

**Parameterization and test of the coupled model of leaf gas-exchange processes.** Using $A-C_{i}$ and light response curves determined on fully elongated young leaves, we parameterized photosynthesis and $g_{s}$ models. The $V_{\text{m25}}$ was estimated at 108.4 $\mu mol\cdot L^{-1}\cdot s^{-1}$ and $J_{\text{m25}}$ was 169.0 $\mu mol\cdot m^{-2}\cdot s^{-1}$. The estimate of $R_{d25}$ was 1.08 $\mu mol\cdot m^{-2}\cdot s^{-1}$. Setting the residual $g_{s}$ ($b$ or $g_{0}$) to 0.096 $mol\cdot m^{-2}\cdot s^{-1}$ as used in Kim and Lieth (2003), the $m$ (or $g_{1}$) parameter for stomatal sensitivity was estimated to be 6.82. The $E_{a}$ for the temperature response of $J_{\text{mmax}}$ was 24.0 $\mu mol\cdot m^{-2}\cdot s^{-1}$, whereas $E_{a}$ for $V_{\text{m}}$ was 52.2 $\mu mol\cdot m^{-2}\cdot s^{-1}$, and $S_{j}$ for $J_{\text{mmax}}$ was estimated to be 616.4 $\mu mol\cdot m^{-2}\cdot s^{-1}$. All other parameter estimates were taken from the literature as listed in Table 1. The calibrated model was capable of following the patterns shown in garlic leaf photosynthetic responses to PPF (Fig. 1A), $CO_{2}$ (Fig. 1B), and temperature (Fig. 1C). Overall, model performance against the calibration data was satisfactory; the model explained 93% of variability in photosynthesis data ($r^{2} = 0.93$) with bias and RMSE of 0.70 and 2.78 $\mu mol\cdot m^{-2}\cdot s^{-1}$, respectively (Fig. 1D). When tested against independent data from Plot 3 that were not used in the parameterization process, the model also demonstrated highly satisfactory performance in photosynthetic response to PPF (Fig. 2A), $CO_{2}$ (Fig. 2B), and temperature (Fig. 2C). The model explained 95% of the
variability (i.e., \( r^2 = 0.95 \)) in photosynthesis of the test data set but with a slight tendency of overestimation with a bias of 1.65 and RMSE of 2.44 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) (Fig. 2D).

The model performance was acceptable in describing transpiration response to temperature and CO2 (Fig. 3A–B). Overall, the coupled model performed to explain 64% of variability in transpiration measurements with an RMSE of 1.02 and bias of -0.24 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) against calibration data. Model performance against the independent test data from Plot 3 was slightly less satisfactory \( (r^2 = 0.49, \text{bias} = -0.14 \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}, \text{RMSE} = 0.94 \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}) \). The model was largely unsuccessful in explaining the variability observed in \( g_s \), especially in response to \( PPFF \) with both calibration and testing data sets (data not shown).

The relationships among chlorophyll meter readings, leaf photosynthetic capacity, and leaf nitrogen content. A positive linear relationship was found between leaf N content and \( A_{\text{max}} \) (Fig. 4A). Likewise, a significant positive correlation was found between leaf N content (grams per square meter) and chlorophyll meter readings \( (r^2 = 0.51; \text{Fig. 4B}) \) with a slope of 0.030 and an intercept of -0.90. Chlorophyll meter readings were also closely related to \( A_{\text{max}} \) \( (r^2 = 0.61) \) indicating that chlorophyll meter readings represented the photosynthetic capacity \( (A_{\text{max}}) \) of garlic leaves reasonably well in this study (Fig. 4C).

**Discussion**

The net CO2 assimilation rates observed in our study for garlic leaves are indicative of a moderate photosynthetic productivity similar to other bulbous or herbaceous \( C_3 \) crops as well as sink capacity of bulb for photosynthates and N (Rosen and Tong, 2001).

The rates of CO2 assimilation at ambient CO2 (\( \approx 400 \mu \text{mol} \cdot \text{mol}^{-1} \)) were similar between leaf temperatures of 15 and 25 °C and gradually declined with increasing temperature above 30 °C (Figs. 1C and 2C). Garlic is a cool-season, hardy perennial crop that is commonly planted in late fall or early winter and harvested in late spring or early summer in temperate regions; it is favored by slightly warmer and drier growth conditions than onions (Kamenetsky, 2007). The photosynthetic response to temperature indicates an adaption to cooler temperatures in ambient CO2. The model predicts that primary limitation in CO2 assimilation under cool temperatures (i.e., less than 13 °C) in saturating light comes from the assimilation rate limited by triose phosphate utilization \( (A_p) \) in both ambient and high CO2 concentrations (Fig. 3C–D). On the other hand, with increasing CO2 \( A \) increases with a clear shift in optimum temperature toward higher temperatures (Fig. 1C). Our model analysis indicates that the rate limitation by Rubisco \((A_c)\) at high temperatures is released in high CO2 (i.e., 1500 \( \mu \text{mol} \cdot \text{mol}^{-1} \)) leaving the regeneration of RuBP limited by electron transport rate \( (A_i) \) to be a sole limiting factor, which resulted in an increase of the apparent temperature optimum for \( A \) (Fig. 3D). This phenomenon is commonly observed in many \( C_3 \) plants in which the competitive inhibition of CO2 assimilation by photorespiration at high temperatures is alleviated in high CO2 (Kim and Lieth, 2003; Sage and Kubien, 2007). The underlying biochemical and physiological mechanisms behind the interactions among temperature, CO2, and light are rather complex but the coupled model was capable of picking up the

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observed interactive patterns in $A$ and $E$ closely (Fig. 3), suggesting its use in studying the future climate impacts in which both $CO_2$ and temperature are expected to rise.

The coupled model was capable of simulating photosynthetic responses of garlic leaves to light, $CO_2$, and temperature with reasonably high accuracy for both calibration and test data sets (Figs. 1 and 2). When compared against independent data that were not used for model calibration, the model performed with a slight tendency to overestimate $A$ and $E$ (Fig. 2). Because the current model predicts “potential” photosynthetic and transpiration rates assuming little or no environmental, biotic, or physiological stress (e.g., insect damage, drought, $N$ deficiency), overestimating the test data from field-grown plants that might have experienced some stressful conditions that are not accounted for by the model is probably more desirable than an underestimation. The strength of a biochemical approach for modeling photosynthesis (Farquhar et al., 1980) as used in the coupled model is in its ability to predict interactions (i.e., $CO_2$, light, and temperature) mechanistically (Kim and Lieth, 2003). This ability to predict interactions among environmental factors (e.g., elevated $CO_2$ and extreme temperatures) that are tightly linked with biochemical and physiological processes such as photosynthetic carbon reduction, photorespiration, and stomatal opening is particularly important for models that are used for climate change research.

Although the coupled model was capable of predicting $A$ with high accuracy under a wide range of environmental conditions (Figs. 1 and 2), it failed to achieve the same accuracy and precision for predicting $g_S$ and transpiration rates (Fig. 3). Similar results have been found in roses for which the coupled model was originally developed (Kim and Lieth, 2003). The coupled model assumes that equilibrium has been reached for both leaf carbon and water balances in each measurement. However, it takes substantially longer for stomata to respond to environmental changes (e.g., lowering light levels) and reach a steady state than the photochemical and biochemical reactions governing $A$ (Kirschbaum et al., 1988). In addition, stomata of some crops have been found to remain open in darkness resulting in considerable nighttime transpiration (Caird et al., 2007). This inability to close stomata in darkness makes it difficult to accurately estimate residual conductance ($b$ or $g_0$) of the $g_S$ model by Ball et al. (1987). In this study, we adopted the estimate of residual conductance from Kim and Lieth (2003) because accurate determination was impractical as a result of large variability. More mechanistic approaches to model $g_S$ (e.g., Buckley et al., 2003) or dynamic $g_S$ models (e.g., Kirschbaum et al., 1988) may improve the ability to predict leaf water balance in a coupled model, but increased complexity with additional parameters and data requirements present challenges in adopting these approaches in current model or crop models in general.

Garlic crop demand for $N$ depends on cultivar, growth stage, soils, and other factors and it continues to increase before bulbing (Rosen and Tong, 2001). During growth, garlic has relatively high demand for $N$ before declining throughout the bulbing phase (Kamenetsky, 2007). The chlorophyll meter readings of well-fertilized plants (46 to 66 SPAD units) observed in our study correspond well with another study that identified an optimal chlorophyll meter reading for maximum biomass gain in garlic at 58.7 SPAD units when 240 kg ha$^{-1}$ of $N$ was supplied (Shin et al., 2005). Our result indicates that relatively high $N$ content (2.8 g m$^{-2}$ or greater) may be required to achieve maximal $A_{max}$ and $A$ is linearly related to leaf $N$ content (Fig. 4A). A linear relationship was also observed between leaf $N$ content and chlorophyll meter readings (Fig. 4B). Likewise, chlorophyll meter readings were also linearly related to leaf $A_{max}$, allowing for prediction of leaf $A_{max}$ as a function of chlorophyll meter readings (Fig. 4C). Our result suggests that chlorophyll meter readings can be a useful rapid non-destructive method to estimate photosynthetic capacity of a garlic leaf. However, it should be noted that there was remaining variability in both leaf $N$ content and $A_{max}$ that was not explained by chlorophyll readings (Fig. 4), suggesting the influence of other factors that may not be captured by a chlorophyll meter.

In summary, we have characterized leaf photosynthetic responses of the hardneck garlic ‘Japanese Mountain’ to $PPF$, $CO_2$, temperature, and leaf senescence. We also parameterized a coupled gas-exchange model to predict garlic photosynthesis and transpiration and tested the model performance using an
independent data set. The coupled model performance was satisfactory with high accuracy for leaf CO₂ assimilation rates but was less effective in predicting transpiration. An improved gs model that mechanistically couples photosynthetic demand with CO₂ supply and leaf water balance will likely improve overall model performance. In addition, we found that chlorophyll meter readings are effective means to estimate photosynthetic capacity in garlic leaves. The leaf level gas-exchange model for garlic leaves presented in this study can serve as a building block of a garlic crop simulation model that is mechanistically based on physiological processes.

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