Photosynthetic and Transpiration Responses to Light, CO₂, 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 = $1.7 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, root mean square error (RMSE) = $2.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$] 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 = $-0.14 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, RMSE = $0.94 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). In addition, we characterized the relationships among chlorophyll meter readings, leaf photosynthetic capacity (A_{max}), and leaf nitrogen content in garlic leaves. The chlorophyll meter readings were a reasonable indicator of both A_{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_{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 ≈ 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, CO₂, 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* (Nielsen 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

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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., 2011)], and *Scaevola aemula* (Kim et al., 2007). Specifically,

the aims of our study were to 1) evaluate photosynthetic responses of garlic leaves to light, temperature, and CO₂; 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/m² 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

Table 1. List of symbols and the estimates of the model parameters used for garlic in the present study.^z

Symbol	Description	Units	Estimate ± SE ^y
A	Leaf net CO ₂ assimilation rate. A is determined by the minimum of A_c , A_j , and A_p minus dark respiration rate (R_d) [i.e., $A = \min(A_c, A_j, A_p) - R_d$]	μmol·m ⁻² ·s ⁻¹	
A_c	Rubisco-limited CO ₂ assimilation rate	μmol·m ⁻² ·s ⁻¹	
A_j	Electron transport-limited CO ₂ assimilation rate	μmol·m ⁻² ·s ⁻¹	
$A_{\max}$	Light saturated A at ambient C_a (≈ 400 μmol·mol ⁻¹)	μmol·m ⁻² ·s ⁻¹	
A_p	Triose phosphate utilization-limited CO ₂ assimilation rate	μmol·m ⁻² ·s ⁻¹	
b	Residual g_s to water vapor. Also known as g_0	mol·m ⁻² ·s ⁻¹	0.096 ^x
C_a	Atmospheric CO ₂ concentration	μmol·mol ⁻¹	
C_i	CO ₂ concentration in intercellular air spaces	μmol·mol ⁻¹	
E	Leaf transpiration rate	mmol·m ⁻² ·s ⁻¹	
g_s	Stomatal conductance to water vapor	mol·m ⁻² ·s ⁻¹	
K_c	Michaelis-Menten constant of Rubisco for CO ₂	μmol·m ⁻² ·s ⁻¹	
K_o	Michaelis-Menten constant of Rubisco for O ₂	μmol·m ⁻² ·s ⁻¹	404.9 ^w
J	Rate of electron transport	μmol·m ⁻² ·s ⁻¹	278.4 ^w
J_{m25}	Potential rate of electron transport at 25 °C	μmol·m ⁻² ·s ⁻¹	169.0 ± 10.30
$J_{\max}$	Potential rate of electron transport	μmol·m ⁻² ·s ⁻¹	
m	Empirical coefficient for the sensitivity of g_s to A , CO ₂ , and relative humidity. Also known as g_1 .	—	6.82 ± 0.36
P_{u25}	Rate of triose phosphate utilization at 25 °C	μmol·m ⁻² ·s ⁻¹	16.03 ± 1.29
R_d	Dark respiration rate. Same values used for day respiration rate.	μmol·m ⁻² ·s ⁻¹	
R_{d25}	R_d at 25 °C	μmol·m ⁻² ·s ⁻¹	1.08 ± 0.13
V_{cm25}	Photosynthetic Rubisco capacity at 25 °C	μmol·m ⁻² ·s ⁻¹	108.4 ± 5.19
$V_{c\max}$	Photosynthetic Rubisco capacity	μmol·m ⁻² ·s ⁻¹	
Γ^*	CO ₂ compensation point in the absence of day respiration	μmol·mol ⁻¹	42.75 ^w
Temperature dependence parameters			
E_a	Activation energy determining exponential rate increase before the peak for:		
	$J_{\max}$	kJ·mol ⁻¹	24.0 ± 2.1
	K_c	kJ·mol ⁻¹	79.43 ^w
	K_o	kJ·mol ⁻¹	36.38 ^w
	P_u	kJ·mol ⁻¹	47.1 ^w
	R_d	kJ·mol ⁻¹	46.39 ^w
	$V_{c\max}$	kJ·mol ⁻¹	52.16 ± 7.1
	Γ^*	kJ·mol ⁻¹	37.83 ^w
H_j	Rate of $J_{\max}$ decrease above the peak temperature	kJ·mol ⁻¹	200 ^v
S_j	Entropy factor for $J_{\max}$	J·K ⁻¹ ·mol ⁻¹	616.4

^zOther model parameters not specified here were used as in Kim and Lieth (2003).

^yAll parameters were determined in this study unless noted otherwise.

^xKim and Lieth (2003).

^wBernacchi et al. (2001).

^vMedlyn 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% NH_4^+ and 7% NO_3^- . Total N supplied during the experiment amounts to $202 \text{ kg} \cdot \text{ha}^{-1}$ (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 $240 \text{ kg} \cdot \text{ha}^{-1}$) (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 CO_2 assimilation (A), stomatal conductance (g_s), 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- CO_2 response (A - C_i) curves were logged at nine reference CO_2 levels: 0, 50, 100, 200, 300, 400, 700, 1000, and $1500 \mu\text{mol} \cdot \text{mol}^{-1}$ 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 $1500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and relative humidity greater than 40%. Photosynthetic light responses (A - Q) were also measured at 11 different PPF levels between 0 and $2000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with reference CO_2 set to $400 \mu\text{mol} \cdot \text{mol}^{-1}$ 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 cm^2) 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 A_{max} 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 A_{max} under saturating light ($1500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), ambient CO_2 ($\approx 400 \mu\text{mol} \cdot \text{mol}^{-2}$), and optimal leaf temperature ($\approx 25^\circ\text{C}$). 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

Fig. 1. Leaf gas-exchange model parameterization for garlic leaves. Measured and predicted net CO_2 assimilation rates (A) for calibration data. (A) Light response at leaf temperature $\approx 24^\circ\text{C}$; (B) A - C_i [internal (CO_2)] response at leaf temperature $\approx 24^\circ\text{C}$; (C) temperature responses at low (square), ambient (circle), and high (triangle) (CO_2), 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 $\pm$ 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 $A_{\max}$ 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 ($V_{\text{cm}25}$), maximum electron transport rate at 25 °C ($J_{\text{m}25}$), rate of triose phosphate utilization at 25 °C ($P_{\text{u}25}$), dark respiration rate at 25 °C ($R_{\text{d}25}$), stomatal sensitivity (m or g_1), activation energy (E_a) for $V_{\text{cm}25}$ and $J_{\text{m}25}$, and entropy factor (S_j) for $J_{\text{m}25}$. 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 $A_{\max}$ at saturating light ($PPF = 2000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in ambient CO₂ (≈ 400 ppm) and air temperature near 25 °C was $23.9 \pm 0.8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 1A). The transpiration rate was $4.67 \pm 0.27 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, g_s was $0.462 \pm 0.043 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the C_i/C_a ratio was 0.75 ± 0.02 for the same conditions (data not shown). The $A-C_i$ response curves revealed that A reached above $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with saturating CO₂ in moderate leaf temperatures (≈ 25 °C); it increased further to $\approx 40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when leaf temperature was raised to near 38 °C in saturating CO₂ ($1500 \mu\text{mol}\cdot\text{mol}^{-1}$) (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 ($\approx 400 \mu\text{mol}\cdot\text{mol}^{-1}$) but shifted to between 35 and 40 °C when CO₂ was elevated to $1500 \mu\text{mol}\cdot\text{mol}^{-1}$

(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 $V_{\text{m}25}$ was estimated at $108.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $J_{\text{m}25}$ was $169.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The estimate of $R_{\text{d}25}$ was $1.08 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Setting the residual g_s (b or g_0) to $0.096 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 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 $J_{\text{m}25}$ was $24.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas E_a for $V_{\text{cm}25}$ was $52.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and S_j for $J_{\text{m}25}$ was estimated to be $616.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. 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), CO₂ (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 $2.78 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 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), CO₂ (Fig. 2B), and temperature (Fig. 2C). The model explained 95% of the

Fig. 2. Leaf gas-exchange model testing for garlic leaves against the observations of net CO₂ assimilation rates (A) that were not used in calibration. (A) Light response at leaf temperature ≈ 24 °C; (B) $A-C_i$ [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 testing. Dashed line represents 1:1 relationship and solid line the regression. All symbols represent mean $\pm$ SE of observations ($n = 1$ for ≈ 40 °C; for others $n = 3$ to 5). Solid lines represent model predictions. RMSE is root mean square error between the predicted (Y) and the observed (X).

Fig. 3. Behavior of garlic leaf gas-exchange model in response to temperature. Modeled temperature response of transpiration (solid line) under atmospheric CO₂ concentration (C_a) = 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ (A) and C_a = 1500 $\mu\text{mol}\cdot\text{mol}^{-1}$ (B) in comparison with observed transpiration rates from calibration data (circle) and testing data (triangle). Modeled temperature response of net CO₂ assimilation rate (A), triose-phosphate use limited rate of CO₂ assimilation (A_p), Rubisco-limited CO₂ assimilation rate (A_c), and electron transport-limited CO₂ assimilation (A_j) under C_a = 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ (C) and C_a = 1500 $\mu\text{mol}\cdot\text{mol}^{-1}$ (D). In the model, A is determined by the minimum of A_c , A_j , and A_p minus dark respiration rate (R_d) [i.e., $A = \min(A_c, A_j, A_p) - R_d$]. All symbols represent mean $\pm$ SE of observations. Lines represent model predictions.

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 $-0.24 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ against calibration data. Model performance against the independent test data from Plot 3 was slightly less satisfactory ($r^2 = 0.49$, bias = $-0.14 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, RMSE = $0.94 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The model was largely unsuccessful in explaining the variability observed in g_s , especially in response to PPF 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_{max} (Fig. 4A). Likewise, a significant positive correlation was found between leaf N content (grams per square meter) and chlorophyll meter readings ($r^2 = 0.51$; Fig. 4B) with a slope of 0.030 and an intercept of -0.90 . Chlorophyll meter readings were also closely related to A_{max} ($r^2 = 0.61$) indicating that chlorophyll meter readings represented the photosynthetic capacity (A_{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 C₃ crops

such as onion (*Allium cepa*) and potato (Fleisher et al., 2010; van Gestel et al., 2005). The A_{max} under saturating light, ambient CO₂, optimal temperature, and adequate water and nutrient availability observed in this study ($23.9 \pm 0.8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) falls within the range of A_{max} found in most C₃ crop plants [i.e., 20 to 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Larcher, 2003)]. The estimates of V_{cm25} and J_{m25} (see Table 1) were also similar to other C₃ crops reported in the literature (Fleisher et al., 2010; Kim et al., 2007; Kim and Lieth, 2003). The photosynthetic capacity parameters, A_{max} , V_{cm25} , and J_{m25} , found in our study were slightly higher than the values reported for onion leaves (van Gestel et al., 2005). Although reported values for garlic photosynthesis in the peer-reviewed literature are limited, Oliveira et al. (2010) reported garlic A_{max} values between 26 and 29 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on a different variety, which are somewhat higher than those found in this study. The A_{max} may vary with cultivars and may also respond to cultural practices such as scape removal, which is likely to alter biomass partitioning

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_j) 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 C₃ 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

Fig. 4. The relationships among photosynthesis, chlorophyll meter (SPAD 502; Konica Minolta, Ramsey, NJ) readings, and leaf nitrogen (N) in garlic leaves. (A) Photosynthetic response (A_{max}) under saturating light, ambient CO_2 , and optimal temperature to leaf nitrogen content; (B) leaf N content and chlorophyll meter readings; and (C) A_{max} and chlorophyll meter readings. Triangles represent data used for model calibration and testing shown in Figures 1, 2, and 3. Open circles were not included in modeling but collected to characterize leaf N, chlorophyll meter readings, and A_{max} relationships.

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 g_s 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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