

Photosynthetic Responses of Swiss Chard, Kale, and Spinach Cultivars to Irradiance and Carbon Dioxide Concentration

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Abstract. The impact of irradiance (0–1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and carbon dioxide concentration (CO₂; 50–1200 ppm) on kale (*Brassica oleracea* and *B. napus pabularia*; three cultivars), Swiss chard (chard, *Beta vulgaris*; four cultivars), and spinach (*Spinacea oleracea*; three cultivars) photosynthetic rate (P_n ; per area basis) was determined to facilitate maximizing yield in controlled environment production. Spinach, chard, and kale maximum P_n were 23.8, 20.3, and 18.2 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ fixed, respectively, across varieties (400 ppm CO₂). Spinach and kale had the highest and lowest light compensation points [LCPs (73 and 13 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively)] across varieties. The light saturation points (LSPs) for chard and kale were similar at 884–978 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, but for spinach, the LSP was higher at 1238 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Dark respiration was lowest on kale and highest on spinach (−0.83 and −5.00 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). The spinach CO₂ compensation point (CCP) was lower (56 ppm) than the chard or kale CCP (64–65 ppm). Among varieties, ‘Red Russian’ kale P_n saturated at the lowest CO₂ concentration (858 ppm), and ‘Bright Lights’ chard saturated at the highest (1266 ppm; 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Spinach P_n was more responsive to increasing irradiance than to CO₂. Kale P_n was more responsive to increasing CO₂ than to irradiance, and chard P_n was equally responsive to increasing CO₂ or irradiance. Implications and limitations of this work when “upscale” to whole-plant responses are discussed.

Leafy green vegetable options are increasing as communities become more ethnically or racially diverse or both, as the health and nutritional benefits of greens consumption are reported (Bertoia et al., 2015; Hu and Rimm, 2015), and as interest in year-round locally produced foods increases (Feldmann and Hamm, 2015). Three increasingly popular leafy vegetables are kale (*Brassica oleracea* and *B. napus pabularia*), spinach (*Spinacea oleracea*), and Swiss chard (chard, *Beta vulgaris*).

Kale, spinach, and chard leaves are harvested and sold on a fresh-weight basis. The ability of plants to increase fresh weight, or mass, is associated with photosynthesis where plant mass generally increases as

photosynthesis increases (Björkman, 1981; Chagvardieff et al., 1994; Dorais, 2003). The primary inputs into the photosynthetic process are light (irradiance), CO₂, and water (Björkman, 1981). Therefore, maximizing photosynthesis in leafy greens to maximize yield would require that irradiance, CO₂, or water not be limited (Fu et al., 2017; Gaudreau et al., 1994; Gent, 2016).

In northern climates, year-round leafy green production requires protected cultivation during the late fall, winter, and early spring when temperatures drop below freezing. Irradiance and CO₂ in protected cultivation often vary, intentionally and unintentionally, depending on covering type, plant spacing, degree of ventilation, whether air is circulated, and whether supplemental lighting or CO₂ are supplied (Kretchten and Howlett, 1970). Little work has been conducted on the effects of irradiance and CO₂ on the P_n of leafy greens other than lettuce (*Lactuca sativa*; Dorais, 2003; Fu et al., 2017; Gaudreau et al., 1994) and recent work by Gent (2016) on spinach. An understanding of how irradiance and CO₂ impact the P_n of such greens would facilitate maximizing the P_n to maximize yield in protected cultivation. The objective of the research presented here was to determine the P_n responses of spinach, kale, and chard to irradiance and CO₂ and to inform producers of the advantages or disadvantages

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of reduced or increased irradiance or CO₂ on yield. We also desired to determine whether leafy green varieties differed in the P_n responses to irradiance and CO₂ and whether some varieties were more suited to supplemental, or reduced, irradiance and CO₂ levels.

We acknowledge that translating instantaneous P_n measurements on a per-unit-area basis to whole-plant photosynthesis has limitations (see Discussion). Yet, an initial comparative study exploring the variation in the instantaneous P_n on a per-unit-area basis is valuable in that it provides some insight into the degree of variation among species and varieties. These data also provide some guidance on irradiance and CO₂ levels that maximize the P_n on the uppermost leaves, especially when plants are young and interior leaf shading is limited.

Materials and Methods

Chard, [‘Rhubarb’, ‘Fordhook Giant’, ‘Bright Yellow’, and ‘Bright Lights’ (red)], kale [‘Toscano’, ‘Winterbor’, and ‘Red Russian’ (*B. napus pabularia*)], and spinach (‘Melody’, ‘Harmony’, and ‘Bloomsdale LS’) seeds were sown in 10.5-cm-diameter plastic pots (5 seeds/pot) in premoistened LC-8 soilless growing media (Sun Gro Horticulture, Bellevue, WA) and placed in a greenhouse (24 ± 2 °C day and 16 ± 2 °C night temperatures; St. Paul, MN). Kale and chard seeds were obtained from Johnny’s Selected Seeds (Winslow, ME), and spinach seeds were obtained from W. Atlee Burpee & Co. (Warminster, PA). Seeds germinated in 4–7 d.

Greenhouse daylight (0800–1400 HR) was supplemented with 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ high-pressure sodium lighting when daylight (at plant level) was below 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. After 7 d, the three most uniform (similar size) seedlings were left to grow, whereas the others were removed. As kale and chard flower after they unfold a specific leaf number, after a cool temperature exposure, or both, daylength was extended with 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 1600–2200 HR as flowering was not a concern [16 h photoperiod; mean daily light integral (DLI) = 12.4 mol·m⁻²·d⁻¹] to simulate a typical production environment to maximize yield (J. Erwin, personal observation). In contrast, as long days can promote flowering on spinach early in development, spinach seedlings were grown under short days (8 h photoperiod; opaque cloth pulled over plants from 1400–0800 HR daily; mean DLI = 10.7 mol·m⁻²·d⁻¹) to inhibit flowering. After 30 d, plants were transplanted into 7.6-l plastic pots. Throughout, plants were watered as needed with irrigation water containing 250 ppm N from 15N–0P–15K fertilizer (Peter’s Dark Weather Feed; The Scotts Co., Marysville, OH). All plants were watered at the same time to ensure similar media nutritional status (confirmed with soil tests) among species and varieties.

Photosynthetic rate determination. After kale plants unfolded seven true leaves (>45° angle from the stem), chard plants unfolded four leaves, and spinach plants unfolded eight

leaves (\approx 4 weeks across species), the impact of irradiance and CO_2 on instantaneous P_n on a per-unit-leaf-area basis was determined on the second leaf below the uppermost fully expanded unfolded leaf on five plants of each species and variety. The P_n was measured using a LI-COR LI6400XT portable photosynthesis meter (LI-COR, Inc., Lincoln, NE) using a cuvette (6 cm²) with a built-in variable LED light source. The P_n at 0, 100, 200, 400, 600, 800, 1000, and 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ irradiance was determined. The P_n at 50, 200, 400, 600, 800, 1000, and 1200 ppm CO_2 was also determined. The P_n was recorded 5 min after a change in irradiance or CO_2 after the P_n had stabilized. Throughout, cuvette temperature was maintained at 24 °C, and the atmospheric flow rate was 400 $\mu\text{L}\cdot\text{min}^{-1}$. Cuvette CO_2 was 400 ppm (outdoor ambient) when determining the P_n responses to irradiance, and irradiance was 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (typical irradiance in a northern U.S. greenhouse during the winter; personal observation) when measuring the P_n responses to CO_2 .

Photosynthetic parameter determination. The P_n data from each leaf of each species and variety at varying irradiance or CO_2 were fit to the nonlinear Mitscherlich and the nonrectangular hyperbola functions as both are widely used to estimate the P_n responses to irradiance and CO_2 (Aleric and Kirkman, 2005; Goudrian, 1979; Johnson et al., 2010; Laitat and Boussard, 1995; Marino et al., 2010; Peek et al., 2002; Potvin et al., 1990). The Mitscherlich equations (Eqs. [1] and [2]) fit data best here and provided realistic nonlinear parameter values (Table 1; personal observation). More complex biochemical models estimating the leaf P_n [such as used by Farquhar et al. (1980)] were not used, as we quantified the P_n responses to CO_2 at irradiance levels typical in northern greenhouses here and not saturating levels typically used with biochemical models.

$$P_n(I) = P_{\max} \left(1 - e^{-k(I-I_0)}\right) \quad \text{Eq. [1]}$$

Eq. [1] shows P_n responses [$P_n(I)$] to irradiance (I). P_n responses to increasing irradiance were asymptotic here; “ P_{\max} ” in Eq. [1] estimates the asymptote. LSP was the irradiance at 95% of P_{\max} . “ I_0 ” was the LCP

(irradiance when estimated $P_n = 0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ CO_2 fixed), and “ k ” was a constant that represented the ratio of the quantum yield (q) to the P_n at the LCP (Marino et al., 2010). R_d (dark respiration) was calculated as the estimated P_n (I) when irradiance was 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

$$P_n(C) = P_{\max} \left(1 - e^{-k(C-C_0)}\right) \quad \text{Eq. [2]}$$

Eq. [2] shows P_n responses [$(P_n(C))$] to CO_2 . P_n responses to increasing CO_2 were asymptotic; “ P_{\max} ” in Eq. [2] estimates the asymptote. “ C_0 ” is the estimated CCP (CO_2 when $P_n = 0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ CO_2 fixed), and “ k ” is a constant. The CO_2 concentration at 95% of P_{\max} approximated the CO_2 saturation point (CSP; CO_2 when P_n was saturated).

Experimental design and data analysis. Analysis of variance (ANOVA) was conducted on photosynthetic parameters derived from Eqs. [1] and [2] fit to P_n data from each leaf on each plant as dependent variables. The experiment was analyzed as a two-stage nested design using Type I sum of squares, which allows the use of Tukey's honestly significant difference (HSD) for mean separation with species as the first factor and cultivar the second. Estimated k values, r^2 , and mean square errors (MSEs) derived from the ANOVA are shown in Table 1. Tukey's HSD ($\alpha < 0.05$) was employed for mean separation except in Table 4 where least significant difference (LSD; $\alpha < 0.05$) was employed as Tukey's HSD cannot be employed with a repeated measures test. A Tukey's test conducted when sphericity is violated (often the case with collected repeated measures tests) will have a vastly inflated Type I error rate; SPSS (see below) presumably does not include Tukey's test as an option under repeated measures analysis to prevent this error. The best test to compare multiple comparisons in a repeated measures design is Bonferroni's; for our data, Bonferroni's gave the same results as LSD. Aside from this case, Tukey's HSD was used when possible as it is more statistically rigorous than LSD. Throughout, the SPSS statistical software package (IBM SPSS Statistics, Version 23; IBM Corp., Armonk, NY) was used for statistical analysis.

Results

Photosynthetic responses to irradiance. P_{\max} (identified by providing saturating irradiance levels at 400 ppm CO_2) differed among species, varieties within a species, and across all varieties (Table 2). Spinach had the highest P_{\max} at 23.8 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ fixed, whereas for kale it was 20.3 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ fixed and for chard it was 18.2 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ fixed (across varieties; Fig. 1; Table 2). Among kale varieties, ‘Red Russian’ and ‘Toscano’ had a higher P_{\max} (21.0–22.3 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) than ‘Winterbor’ (17.4 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; Table 2). Among spinach varieties, ‘Melody’ had a higher P_{\max} (26.6 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) than ‘Harmony’ or ‘Bloomsdale LS’ (22.3–22.4 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; Table 2). Among chard varieties, ‘Fordhook Giant’ had a higher P_{\max} (21.9 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) than ‘Bright Lights’ or ‘Yellow’ (16.3–16.6 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; Table 2). Across all species and varieties, ‘Melody’ spinach had the highest P_{\max} , and ‘Yellow’ and ‘Bright Lights’ chard had the lowest P_{\max} (Table 2).

Spinach had the highest LCP (73 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the LCP of chard was 25 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and kale had the lowest LCP at 13 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Table 2). Kale and spinach variety LCP did not differ, but chard variety LCP differed from 16 (Bright Lights) to 41 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Rhubarb; Table 2). Chard and kale LSP (884–978 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) did not differ, but spinach LSP was higher (1238 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; Table 2). LSP did not differ among varieties within any species studied here (Table 2).

Photosynthetic responses to CO_2 concentration. P_{\max} (identified by providing saturating CO_2 concentrations at 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) differed among some species, among varieties within some species, and across all varieties (Table 3). Chard and kale P_{\max} did not differ (17.2–17.6 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ fixed), but spinach P_{\max} was higher at 19.8 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ fixed (across varieties; Fig. 1; Table 3). P_{\max} did not differ among spinach varieties. Among kale varieties, ‘Winterbor’ had a lower P_{\max} than ‘Red Russian’ (Table 3). Chard P_{\max} varied from 16.6 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for ‘Rhubarb’ to 19.2 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for ‘Bright Lights’ (Table 3). Across varieties,

Table 1. Statistics associated with goodness of fit of raw data to the nonlinear Mitscherlich function used to describe responses of kale (*Brassica oleracea* and *B. napus pubularia*; three cultivars), spinach (*Spinacea oleracea*; three cultivars), and Swiss chard (*Beta vulgaris*; four cultivars) to irradiance and carbon dioxide concentration. The r^2 , mean square error (MSE), and “ k ” values (Mitscherlich function) fit to data are shown.

Plant type	Irradiance			Carbon dioxide concn		
	k	r^2	MSE	k	r^2	MSE
Kale	0.0031	0.996	0.157	0.0033	0.998	0.122
‘Red Russian’	0.0030	0.999	0.059	0.0038	0.998	0.149
‘Winterbor’	0.0034	0.991	0.234	0.0028	0.999	0.048
‘Toscano’	0.0030	0.998	0.180	0.0032	0.998	0.168
Spinach	0.0027	0.999	0.038	0.0032	0.999	0.068
‘Melody’	0.0026	0.999	0.058	0.0034	0.999	0.056
‘Harmony’	0.0028	1.000	0.027	0.0032	0.999	0.064
‘Bloomsdale LS’	0.0026	0.999	0.030	0.0030	0.999	0.083
Swiss chard	0.0036	0.998	0.099	0.0030	0.998	0.177
‘Yellow’	0.0040	0.999	0.051	0.0030	0.993	0.524
‘Rhubarb’	0.0034	0.997	0.165	0.0035	1.000	0.016
‘Fordhook Giant’	0.0030	0.999	0.088	0.0030	1.000	0.023
‘Bright Lights’	0.0040	0.998	0.093	0.0026	0.999	0.112

Table 2. Variation in predicted maximum photosynthetic rate (P_{\max}), the light compensation point (irradiance at $P_n = 0$), the light saturation point (irradiance at 95% of P_{\max}), predicted dark respiration rate (CO_2 evolution in dark at 24 °C), and quantum efficiency among three varieties of kale (*Brassica oleracea* and *B. napus pabularia*), three varieties of spinach (*Spinacea oleracea*), and four varieties of Swiss chard (*Beta vulgaris*) as determined using a fitted Mitscherlich model for each plant of each species and variety. Capital letters denote mean separation [Tukey's HSD(0.05)] across species, and small letters denote mean separation across varieties.

Plant species/variety	Maximum photosynthetic compensation rate ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Light saturation point ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Light respiration point ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Dark efficiency rate ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Quantum
Kale	20.3 B	13 A	978 A	-0.83 C	0.07 A
‘Red Russian’	22.3 ± 0.5 d	16 ± 2 ab	1,015 ± 2	-1.12 ± 0.12 c	0.07 ± 0.002
‘Toscano’	21.0 ± 1.1 bcd	15 ± 2 ab	1,014 ± 2	-0.97 ± 0.11 c	0.06 ± 0.004
‘Winterbor’	17.4 ± 0.7 ab	6 ± 1 a	905 ± 61	-0.39 ± 0.10 c	0.06 ± 0.004
Spinach	23.8 C	73 C	1,238 B	-5.00 A	0.08 B
‘Melody’	26.6 ± 0.7 e	68 ± 3 e	1,266 ± 123	-5.05 ± 0.44 a	0.08 ± 0.007
‘Harmony’	22.4 ± 1.2 d	79 ± 3 e	1,177 ± 102	-5.51 ± 0.43 a	0.08 ± 0.006
‘Bloomsdale LS’	22.3 ± 0.8 d	72 ± 4 e	1,270 ± 126	-4.46 ± 0.23 a	0.07 ± 0.007
Swiss chard	18.2 A	25 B	884 A	-1.64 B	0.07 AB
‘Bright Lights’	16.6 ± 0.8 a	16 ± 2 ab	784 ± 64	-10.6 ± 0.13 c	0.07 ± 0.003
‘Fordhook Giant’	21.9 ± 0.7 cd	22 ± 2 b	1,021 ± 17	-1.49 ± 0.11 bc	0.07 ± 0.002
‘Rhubarb’	18.0 ± 0.8 abc	41 ± 4 c	939 ± 65	-2.60 ± 0.20 b	0.07 ± 0.004
‘Yellow’	16.3 ± 0.9 a	21 ± 4 b	790 ± 65	-1.43 ± 0.31 bc	0.07 ± 0.004
Analysis of Variance					
Species	***	***	***	***	*
Variety	***	***	NS	***	NS

HSd = honestly significant difference; ns = nonsignificant.

^aDenotes significance at the $\alpha < 0.05$ (*), < 0.01 , (**), < 0.001 (***) levels.

‘Winterbor’ kale had the lowest P_{\max} (14.7 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and ‘Red Russian’ and ‘Toscano’ kale had the highest P_{\max} (17.8 and 19.0 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively; Table 3).

Spinach CCP was lower (56 ppm) than chard or kale CCPs (64–65 ppm) across varieties (Table 3). CCP did not differ among spinach or chard varieties, but differed among kale varieties where ‘Red Russian’ had the lowest CCP (59 ppm) and ‘Winterbor’ the highest (72 ppm; Table 3). CSP did not vary among species or among varieties within a species, but differed when all varieties were compared; ‘Red Russian’ kale had the lowest CSP (858 ppm), and ‘Bright Lights’ chard had the highest CSP (1266 ppm; Table 3).

Dark respiration. Calculated R_d (24 °C) differed among species. Kale R_d was the lowest ($-0.83 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), chard R_d was $-1.64 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, and spinach R_d was the highest ($-5.00 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; across varieties; Table 2). R_d did not differ among kale and spinach varieties, but differed among chard varieties where ‘Rhubarb’ and ‘Bright Lights’ chard R_d was -2.60 and $-10.06 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively (Table 2).

Discussion

Limitations of generalizing instantaneous P_n data on a per-unit-area basis to whole-plant P_n . Data here provide a framework for determining irradiance and CO_2 impacts on kale, spinach, and chard P_n to facilitate production in controlled environment facilities to maximize yield. P_n is often associated with dry weight gain, fresh weight gain, and yield in vegetables (Heuvelink and Dorais, 2003). There are limitations when extrapolating changes in instantaneous P_n measurements on a per-unit-area basis to whole-plant P_n and conclusions drawn from that data. For instance, instantaneous P_n responses can

differ from whole-plant responses when a) multiple inputs are changed at once, b) after plants acclimate to altered irradiance, CO_2 , or both, and c) when leaf aging and whole-plant leaf area/shading are taken into account.

One environmental parameter (irradiance or CO_2) was changed while the other was held constant in our research here. Increasing irradiance and CO_2 simultaneously may produce different conclusions, likely increasing LSP, CSP, or both more than reported here. For instance, Chagvardieff et al. (1994) observed increasing CO_2 and irradiance simultaneously increased lettuce dry weight 69% more than dry weight gains observed from increasing CO_2 and irradiance separately, i.e., there was a synergy between these factors. Also, other environmental parameters can interact with irradiance, CO_2 , or both to impact P_n . Changes in humidity (Kaiser et al., 2015) or temperature (Dahal et al., 2012) can result in markedly different responses in P_n to irradiance, CO_2 , or both. Nonenvironmental cultural factors can also impact P_n and assumptions made here. For instance, high irradiance promotion of P_n was most obvious when lettuce was grown under low nitrogen levels only (7 $\text{mmol} \cdot \text{L}^{-1}$; Fu et al., 2017).

Photosynthetic rate can acclimate to altered irradiance or CO_2 over time (Björkman, 1981; Lambers et al., 2008; Pons, 2012). Therefore, caution should be exercised when extrapolating instantaneous P_n responses to whole-plant P_n over time. P_n at high irradiance or CO_2 may be overpredicted, and P_n at low irradiance or CO_2 may be underpredicted over time (Bunce and Ziska, 2000). The basis for P_n acclimation to altered irradiance or CO_2 is not clear. Acclimation of P_n to high irradiance or CO_2 was more correlated with soluble saccharides than with day to day variation in CO_2 or irradiance alone (Bunce and Sicher, 2003). In contrast, variation among *Arabidopsis* varieties in P_n over time

to irradiance was associated with differences in Rubisco activation and stomatal conductance (g_S) (Kaiser et al., 2016).

Also, the transferability of our conclusions to whole-plant responses is associated with the size (or age) of a plant and leaf area. High irradiance can result in reduced leaf life or smaller leaf area which can result in an overestimating whole-plant P_n if reduced leaf area, more rapid leaf senescence, or both is not taken into account (Austin, 1989). Also, although P_n on the uppermost leaf may be saturated, whole-plant P_n is likely not saturated as lower leaves are shaded in a canopy as a plant grows and unfolds leaves. Such shading results in P_n rates lower than the P_{\max} on lower leaves even when irradiance on the uppermost leaves is at the LSP. Irradiance in a canopy decreases exponentially from the top to the bottom of a plant following the general equation $I = I_0 e^{-kL}$ [I = irradiance below the canopy; I_0 = irradiance at the top of the canopy; k = the extinction coefficient (generally > 0.5 for nonvertically oriented leaves); and L = leaf area index (Lambers et al., 2008)]. Therefore, increasing irradiance at the top of the plant above the LSP will likely increase whole-plant P_n if the leaf area index is high.

Although these limitations when translating instantaneous P_n data to crop responses exist, we believe P_n responses are still informative. Specifically, instantaneous P_n data on a per-unit-area basis provide insight into variation in responses among species and varieties that is of value and provide some insight into which species or varieties may be more responsive to increases in irradiance or CO_2 concentration.

Responses to irradiance. Kale and chard P_n saturated at lower (600–800 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) irradiance levels than spinach (1000–1200 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; 400 ppm CO_2 ; Fig. 1; Table 2). P_{\max} reported here are consistent with previous data on spinach (Boese and Huner, 1990; Yamori et al., 2005) and kale

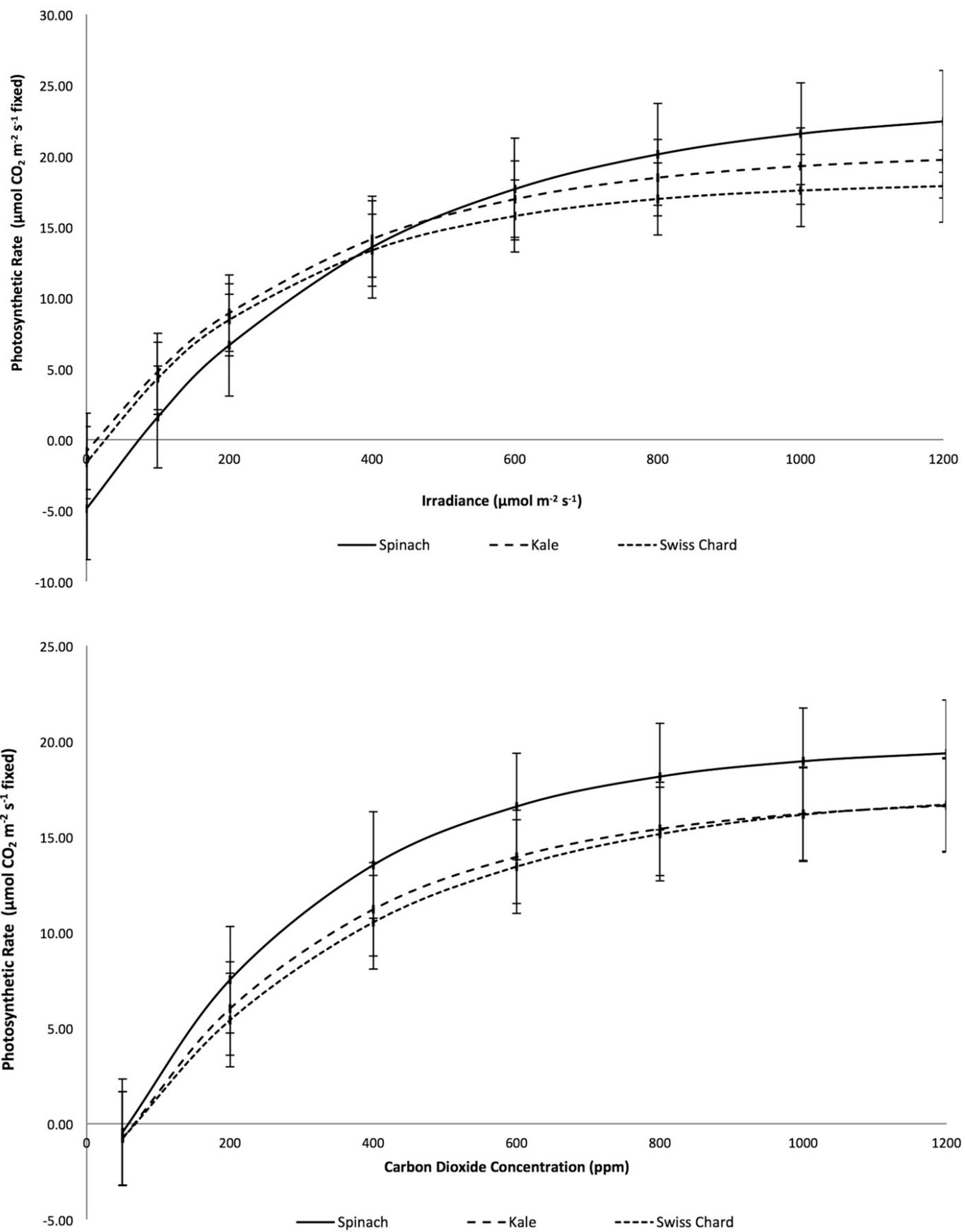


Fig. 1. Effect of increasing irradiance (A) or carbon dioxide (CO₂) concentration (B) on spinach (*Spinacea oleracea*), kale (*Brassica oleracea* and *B. napus* *pabularia*), and Swiss chard (*Beta vulgaris*) photosynthetic rate across cultivars. Bars represent the \pm mean square error as identified through analysis of variance ($\alpha < 0.05$).

Table 3. Variation in the predicted maximum photosynthetic rate (P_{\max} ; $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), the CCP (CO₂ concentration when $P_n = 0$), and the CSP (the CO₂ concentration at 95% P_{\max}) among three cultivars of kale (*Brassica oleracea* and *B. napus pabularia*), three cultivars of spinach (*Spinacea oleracea*), and four cultivars of Swiss chard (*Beta vulgaris*) as determined using fitted Mitscherlich functions fit to each cultivar and pooled under each species. Capital letters denote mean separation [Tukey's HSD(0.05)] across species, and small letters denote mean separation across cultivars.

Plant species/cultivar	P_{\max} ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	CCP (ppm)	CSP (ppm)
Kale	17.2 A	65.0 B	1,014
‘Red Russian’	17.8 + 0.5 bcde	58.8 + 1.5 abc	858 + 51 a
‘Toscano’	19.0 + 0.3 cde	64.4 + 2.1 bcd	1,013 + 51 ab
‘Winterbor’	14.7 + 0.5 a	71.8 + 3.1 d	1,170 + 101 ab
Spinach	19.8 B	56.1 A	1,005
‘Melody’	19.8 + 0.2 de	54.8 + 0.8 a	954 + 61 ab
‘Harmony’	19.6 + 0.3 de	55.6 + 1.0 ab	1,004 + 51 ab
‘Bloomsdale LS’	20.0 + 0.4 e	58.0 + 1.1 abc	1,057 + 11 ab
Swiss chard	17.6 A	64.3 B	1,102
‘Bright Lights’	19.2 + 0.6 cde	67.3 + 2.7 cd	1,266 + 124 b
‘Fordhook Giant’	17.5 + 0.7 bcd	64.1 + 3.1 abcd	1,113 + 125 ab
‘Rhubarb’	16.6 + 0.9 ab	63.0 + 1.9 abcd	939 + 73 ab
‘Yellow’	16.9 + 0.6 abc	62.8 + 0.7 abcd	1,061 + 74 ab
Analysis of Variance			
Species	***	***	NS
Cultivar	***	**	*

P_{\max} = maximum photosynthetic rate; CCP = CO₂ compensation point; CSP = CO₂ saturation point; HSD = honestly significant difference; NS = nonsignificant.

Denotes significance at the $\alpha < 0.05$ (), < 0.01 , (**), < 0.001 (***) levels.

[*Brassica*; Dahal et al. (2012) (*napus*) and Ruhil et al. (2015)] at slightly lower CO₂ concentrations than used here. Given irradiance in northern climates in greenhouses rarely exceeds LSPs reported here (personal observation), supplemental lighting (up to the LSPs, at a minimum) would increase P_n and presumably yield. Supplemental lighting in northern greenhouse vegetable production facilities is commonplace as growers observe increased yield when providing supplemental light. In greenhouse lettuce production, daylight is often supplemented with 50–100 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (DLI = 12–13 mol·m⁻²·d⁻¹) during the winter to realize 140% to 270% increases in yield compared with plants with no supplemental lighting in Canada (Gaudreau et al., 1994). Similarly, Brassicaceae microgreen (seedling) fresh weight and nutritional value increased when irradiance was increased from 0 to 320–440 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, but not when irradiance was further increased from 440 to 545 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Samuoliene et al., 2013). In contrast, Colonna et al. (2016) showed that the impact of irradiance on the nutritional value of 10 leafy vegetables varied with species.

Daily light integral can be more associated with yield than with irradiance as it represents the cumulative light delivered over 24 h. For instance, spinach dry weight as a ratio of fresh weight increased as normalized daily light integral (DLI/leaf area index) increased from 3 to 27 mol·m⁻²·d⁻¹ (Gent, 2016). However, it is important to note that a high correlation between DLI and plant dry weight will occur only if irradiance is below the LSP; irradiance levels above the LSP would not result in an increase in P_n .

Based on the instantaneous P_n data here, kale and chard may be better suited for production in naturally low irradiance locations or facilities than spinach as their LSP were lower than that of spinach (Table 2). Also, as spinach had a higher LCP (60–75 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) than kale (10–15 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), kale may be grown under lower irradiance conditions than spinach and still have a net increase in mass. Again, we emphasize that

these assumptions are based on instantaneous data, and plants may acclimate to lower irradiance levels over time.

Table 4 shows predicted percent changes in P_n when irradiance was increased from 300 to 350 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ on kale, chard, and spinach (DLI = +3.24 mol·m⁻²·d⁻¹ for chard and kale, and +1.44 mol·m⁻²·d⁻¹ for spinach as photoperiod differed). Increasing irradiance from 300 to 350 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ increased predicted spinach P_n by 15% and that of kale and chard by 9% to 11% (Table 4).

Responses to CO₂. Kale and chard CSP was lower (600–800 ppm) than that of spinach (1000–1200 ppm) (irradiance = 300 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; Fig. 1; Table 3). Responses observed on kale here were similar to those observed by others (700 ppm CO₂) on *B. oleracea* and *B. napus* under different irradiance levels (Bunce and Sicher, 2003; Dahal et al., 2012, respectively). Given all CSPs reported here are higher than the ambient CO₂ levels, supplementing greenhouses or growth rooms with CO₂ (above ambient) would likely increase the P_n of crops studied here (Table 3). In fact, injecting CO₂ to increase CO₂ levels to 800–1000 ppm is commonplace in commercial vegetable production greenhouses to increase yield (personal observation; Dorais, 2003). For instance, increasing CO₂ from 330 to 900 ppm increased tomato yield by 21% (Dorais, 2003).

P_n in unventilated greenhouses or enclosed rooms can be limited by declining CO₂ levels as plants use CO₂ for photosynthesis. It is not uncommon for CO₂ levels in a canopy to drop to 200–250 ppm during the day in an unvented greenhouse or growth room (J. Erwin and J. Frantz, personal observations). Therefore, ventilating production environments with outdoor air (400 ppm CO₂) will increase CO₂ concentration inside thus increasing P_n . Our data predict ventilating greenhouses to increase CO₂ levels from 200 to 400 ppm can result in a greater increase in P_n than that from increasing CO₂

from 400 to 800 ppm as would occur when using a CO₂ injection system (Table 4). For instance, increasing CO₂ from 200 to 400 ppm increased predicted P_n by 75% to 98% across species whereas increasing CO₂ levels from 400 ppm to the CSP increased predicted P_n by 38% to 68% across species (Table 4). These data emphasize the importance of ventilating to ensure canopy CO₂ levels are at least similar to ambient outdoor levels. Of course, the predicted increases in P_n if CO₂ is increased from 400 to 800 ppm may be greater if irradiance was simultaneously increased to the LSP.

As with P_n responses to irradiance, although CO₂ levels on the uppermost leaves may be at CSP levels, lower leaf P_n is likely not at CSP levels as CO₂ is consumed by photosynthesis in the canopy and replaced slowly (depending on air circulation and ventilation). Therefore, increasing CO₂ levels to above the CSP often results in increased yields as lower leaf P_n is less CO₂ limited. This is increasingly important as leaf area index increases in a canopy. Kale and chard had lower CSP than spinach; therefore, these crops would perform better in greenhouses with poor ventilation or with close plant spacing than spinach (Table 3).

Respiration. Spinach, kale, and chard predicted R_d (24 °C) observed here are comparable with those measured by others although temperatures differed somewhat (Dahal et al., 2012; Yamori et al., 2005). We observed variation in R_d among species and among varieties of some species. Although spinach variety R_d was high and similar, kale and chard variety R_d varied with some varieties having a 4-fold higher R_d than with other varieties (Table 2). Variation in R_d among varieties of other vegetable species (such as asparagus) has also been observed (Kitazawa et al., 2011). Such variation in R_d among species and varieties here are especially important to quantify as R_d can be negatively correlated with postharvest performance (Kitazawa et al., 2011). For

Table 4. Predicted instantaneous photosynthetic rates (P_n per unit area; $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) at low irradiance (typical cloudy day in winter; 200 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), ambient irradiance (typical 300 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and at 95% of predicted P_{\max} (P_n saturated) of kale (*Brassica oleracea* and *B. napus pabularia*), Swiss chard (*Beta vulgaris*), and spinach (*Spinacea oleracea*) varieties. Predicted P_n at low CO_2 levels (200 ppm; depleted enclosed environment), at ambient CO_2 levels (400 ppm, current outdoor), and at 95% of predicted P_{\max} of kale, Swiss chard, and spinach varieties. Percentage in parenthesis is the percent increase in P_n when increasing from the rate one line up and to the left, to the level above the number in parentheses. Letters denote mean separation [Tukey's HSD ($\alpha < 0.05$)] within a variety to increasing irradiance or increasing carbon dioxide concentration.

Species and cultivar	Irradiance ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)					Carbon dioxide (ppm)			
	Low 200	Ambient 300	+50 350	P_n Saturated	600–1,000	Deficient 200	Ambient 400	P_n Saturated	800–1,000
Kale									
'Red Russian'	9.46 b	12.80 c (+35%)	14.13 d (+10%)	22.34 f (+75%)	7.37 a	12.87 c (+75%)	17.81 e (+38%)		
'Toscano'	8.96 b	12.10 c (+35%)	13.34 d (+10%)	21.04 e (+74%)	6.66 a	12.44 cd (+87%)	18.98 e (+53%)		
'Winterbor'	8.33 b	10.90 c (+31%)	11.89 d (+9%)	17.41 f (+60%)	4.42 a	8.77 b (+98%)	14.72 e (+68%)		
Spinach									
'Melody'	7.66 a	11.89 b (+55%)	13.63 c (+15%)	26.58 e (+124%)	7.70 a	13.61 bc (+77%)	19.83 d (+46%)		
'Harmony'	6.37 a	10.22 b (+60%)	11.78 c (+15%)	22.45 d (+120%)	7.24 a	13.04 c (+80%)	19.59 d (+50%)		
'Bloomsdale LS'	6.27 a	9.85 b (+57%)	11.31 c (+15%)	22.27 e (+126%)	6.94 a	12.84 c (+85%)	20.00 d (+56%)		
Chard									
'Yellow'	8.21 b	10.81 c (+32%)	11.77 d (+9%)	16.35 e (+53%)	5.70 a	10.75 c (+88%)	16.89 e (+63%)		
'Rhubarb'	7.55 a	10.52 b (+39%)	11.67 c (+11%)	17.87 e (+70%)	6.23 a	11.33 bc (+82%)	16.55 d (+46%)		
'Fordhook Giant'	9.05 b	12.37 c (+37%)	13.69 d (+11%)	21.86 f (+77%)	5.76 a	10.87 c (+89%)	17.50 e (+61%)		
'Bright Lights'	8.54 b	11.12 cd (+30%)	12.08 d (+9%)	16.62 fe (+50%)	5.53 a	10.92 c (+97%)	19.17 f (+76%)		

HSD = honestly significant difference.

instance, data here suggest that spinach may have a shorter postharvest life than kale and that among kale varieties and among chard varieties, 'Bright Lights' may have a shorter postharvest life than 'Rhubarb' (Table 2).

Irradiance versus CO_2 . The question arises as to whether a spinach, kale, or chard producer should increase irradiance or CO_2 to increase yield most. Low CO_2 conditions (200 ppm) reduced predicted P_n more than low irradiance ($200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) conditions on both kale and chard here (Table 4). Therefore, ventilating enclosed production spaces (where CO_2 may have dropped to 200 ppm) to ensure CO_2 levels are at ambient levels (400 ppm) may increase P_n and likely yield on kale and chard more than on spinach. In contrast, in nearly all cases (except 'Toscano' kale, and 'Yellow' and 'Bright Lights' chard), increasing irradiance from ambient irradiance ($300 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) to the LSP increased predicted P_n more than increasing CO_2 from ambient (400 ppm) to the CSP (Table 4). This suggests that irradiance may be more limiting than CO_2 with the crops studied here.

Future work must examine the synergy between irradiance and CO_2 on kale, chard, and spinach. Increasing CO_2 from 400 to 800 ppm and increasing irradiance from 400 to 800 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ significantly increased lettuce dry weight by 25% (1.5 g/plant) and 19%, (1.15 g/plant), respectively (Chagvardieff et al., 1994). However, increasing CO_2 concentration and irradiance simultaneously acted synergistically [2.65 g/plant when benefits added individually vs. 4.21 g/plant (+69%) when increased together] when conducted 23 to 40 d after sowing (Chagvardieff et al., 1994).

Variety differences. Varieties (of some species) differed in response to increasing irradiance or CO_2 suggesting different genetic backgrounds. There was little variation among spinach varieties in P_{\max} , LCP, CCP, LSP, and CSP (Tables 1 and 2). However, there were substantial differences among kale and chard varieties for these same

parameters (Tables 1 and 2). This was not unexpected as vegetable crops are often interspecific hybrids, and varieties can vary greatly genetically. Similar variation in P_{\max} among lettuce varieties was observed by Behr and Wiebe (1992). Gu et al. (2012) observed variation in P_{\max} (17% to 25% variation) in rice (13 lines; *Oryza sativa* L.) at ambient CO_2 levels (380 ppm) and that variation was associated with stomatal and mesophyll conductance. Yu et al. (2016) found variation in *Cucumis* P_n varieties to changes in irradiance was associated with differences in leaf chlorophyll content. In another work, differences in *Arabidopsis* variety P_n responses to changing irradiance was associated with differences in Rubisco activation and g_S (Kaiser et al., 2016). Whatever the basis, our data infer genetic diversity (based on P_n responses) of kale and chard may be greater than that of the spinach varieties studied here.

Combining both P_n and R_d . It cannot also be assumed that higher P_n will result in increased fresh or dry weight and yield as yield is associated with carbon loss or R_d . As R_d occurs during both day and night, it can have a significant negative impact on net daily carbon gain. When predicted daily carbon gain was calculated by taking both P_n (R_d already quantified in direct P_n readings) and R_d (at night only) into account {net carbon gain = $[(P_{\max} \times 18 \text{ h} \cdot \text{d}^{-1}) (\text{kale}) \text{ or } 8 \text{ h} \cdot \text{d}^{-1} (\text{spinach})] - [(R_d \times 8 \text{ h} \cdot \text{d}^{-1}) (\text{kale}) \text{ or } 16 \text{ h} \cdot \text{d}^{-1} (\text{spinach})]$ }, we observed spinach carbon gain/d ($380.8 - 80.0 = 300.8 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) was lower than that of kale ($365.4 - 6.6 = 358.8 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) even though P_{\max} was greater (Table 2).

Take home messages.

1. Kale and chard P_n saturated at lower ($600-800 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) irradiance levels than spinach P_n ($1000-1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and kale and chard may be better suited for production in low-irradiance facilities than spinach. Also, as spinach had a higher LCP

($60-75 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) than kale ($10-15 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), kale may be grown under lower irradiance conditions than spinach and still have an increase in mass.

2. Kale and chard CSP were lower (600–800 ppm) than that of spinach (1000–1200 ppm). Given all CSPs reported here are higher than ambient CO_2 levels, supplementing CO_2 would increase the P_n of crops studied here.
3. Ventilating greenhouses to increase CO_2 from 200 to 400 ppm may result in a greater increase in P_n than that from increasing CO_2 from 400 to 800 ppm.
4. Although spinach variety R_d was similar, kale and chard variety R_d varied with some varieties having a 4-fold higher R_d than others.
5. Low CO_2 reduced kale and chard P_n more than low irradiance. Therefore, ventilating production spaces to ensure CO_2 levels are at 400 ppm may increase yield more on kale and chard than on spinach.
6. In nearly all cases (except 'Toscano' kale, and 'Yellow' and 'Bright Lights' chard), increasing irradiance from 300 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to the LSP increased the P_n more than increasing CO_2 from ambient to the CSP. This suggests irradiance may be more limiting than CO_2 on these crops.
7. When predicted daily carbon gain was calculated by taking both P_n and R_d into account, spinach carbon gain per day was lower than that of kale even though the P_{\max} was greater.

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