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 μmol·m−2·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 (Pn; per area basis) was determined to facilitate maximizing yield in controlled environment production. Spinach, chard, and kale maximum photosynthetic rate (C176) were similar across species and varieties (400 ppm CO2). Spinach and kale had the highest and lowest light compensation points [LCPs (73 and 13 μmol·m−2·s−1, respectively)] across varieties. The light saturation points (LSPs) for chard and kale were similar across species (884–978 μmol·m−2·s−1, but for spinach, the LSP was higher across species (1238 μmol·m−2·s−1). Dark respiration was lowest across species and varieties. The spinach CO2 compensation point (CCP) was lower (56 ppm) than the chard or kale CCP (64–65 ppm). Among varieties, ‘Red Russian’ kale Pn was saturated at the lowest CO2 concentration (858 ppm), and ‘Bright Lights’ chard saturated at the highest (1266 ppm) across species; 300 μmol·m−2·s−1). Spinach Pn was more responsive to increasing irradiance than to CO2. Kale Pn was more responsive to increasing CO2 than to irradiance, and chard Pn was equally responsive to increasing CO2 or irradiance. Implications and limitations of this work when “upsizing” 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; Doraï, 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, depth of vegetation, whether air is circulated, and whether supplemental lighting or CO2 are supplied (Kretchen and Gaudreau, 1994; Gent, 2016). 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 1400–2200 h) as flowering is not a concern [16 h photoperiod; mean daily light integral (DLI) = 12.4 μmol·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 1400–2200 h) as flowering was not a concern [16 h photoperiod; mean daily light integral (DLI) = 12.4 μmol·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 1400–2200 h) as flowering was not a concern [16 h photoperiod; mean daily light integral (DLI) = 12.4 μmol·m−2·d−1] to simulate a typical production environment to maximize yield (J. Erwin, personal observation).
leaves (≈4 weeks across species), the impact of irradiance and CO₂ on instantaneous Pₙ 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 Pₙ 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 Pₙ at 0, 100, 200, 400, 600, 800, 1000, and 1200 μmol·m⁻²·s⁻¹ irradiance was determined. The Pₙ at 50, 200, 400, 600, 800, 1000, and 1200 ppm CO₂ was also determined. The Pₙ was recorded 5 min after a change in irradiance or CO₂ after the Pₙ had stabilized. Throughout, cuvette temperature was maintained at 24 °C, and the atmospheric flow rate was 400 μL·min⁻¹. Cuvette CO₂ was 400 ppm (outdoor ambient) when determining the Pₙ responses to irradiance, and irradiance was 300 μmol·m⁻²·s⁻¹ (typical irradiance in a northern U.S. greenhouse during the winter; personal observation) when measuring the Pₙ responses to CO₂.

Photosynthetic parameter determination. The Pₙ data from each leaf of each species and variety at varying irradiance or CO₂ were fit to the nonlinear Mitscherlich and the nonrectangular hyperbola functions as both fit to the nonlinear Mitscherlich and the synthesis meter (LI-COR, Inc., Lincoln, NE) (LCP).

Photosynthetic responses to irradiance. 'Bright Lights' 0.0040 0.998 0.093 0.0026 0.999 0.112
'Bloomsdale LS' 0.0026 0.999 0.030 0.0030 0.999 0.083
'Green' 0.0030 0.999 0.058 0.0034 0.999 0.056
'Toscano' 0.0030 0.998 0.180 0.0032 0.998 0.168
'Winterbor' 0.0034 0.991 0.234 0.0028 0.999 0.048

Photosynthetic responses to CO₂ concentration. Chard had the highest LCP (73 μmol·m⁻²·s⁻¹), the LCP of chard was 25 μmol·m⁻²·s⁻¹, and kale had the lowest LCP at 13 μmol·m⁻²·s⁻¹ (Table 2). Kale and spinach variety LCP did not differ, but chard variety LCP differed from 16 (Bright Lights) to 41 μmol·m⁻²·s⁻¹ (Rhubarb; Table 2). Chard and kale LCP (884–978 μmol·m⁻²·s⁻¹) did not differ, but spinach LSP was higher (1238 μmol·m⁻²·s⁻¹; Table 2). Among all species and varieties, 'Melody' spinach had the highest Pₘₕₐₓ and 'Yellow' and 'Bright Lights' chard had the lowest Pₘₕₐₓ (Table 2).

Photosynthetic responses to CO₂ concentrations at 300 μmol·m⁻²·s⁻¹ differed among some species, among varieties within some species, and across all varieties (Table 3). Chard and kale Pₘₕₐₓ did not differ (17.2–17.6 μmol·m⁻²·s⁻¹ fixed), but spinach Pₘₕₐₓ was higher at 19.8 μmol·m⁻²·s⁻¹ fixed (across varieties; Fig 1; Table 2). Among kale varieties, 'Red Russian' and 'Toscana' had a higher Pₘₕₐₓ (21.0–22.3 μmol·CO₂·m⁻²·s⁻¹) than 'Winterbor' (17.4 μmol·CO₂·m⁻²·s⁻¹; Table 2). Among spinach varieties, 'Melody' had a higher Pₘₕₐₓ (26.6 μmol·CO₂·m⁻²·s⁻¹) than 'Harmony' or 'Bloomdale LS' (22.3–22.4 μmol·CO₂·m⁻²·s⁻¹; Table 2). Among chard varieties, 'Fordhook Giant' had a higher Pₘₕₐₓ (21.9 μmol·CO₂·m⁻²·s⁻¹) than 'Bright Lights' or 'Yellow' (16.3–16.6 μmol·CO₂·m⁻²·s⁻¹; Table 2). Across all species and varieties, 'Melody' spinach had the highest Pₘₕₐₓ and 'Yellow' and 'Bright Lights' chard had the lowest Pₘₕₐₓ (Table 2).

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Table 2. Variation in predicted maximum photosynthetic rate ($P_{\text{max}}$), the light compensation point (irradiance at $P_n = 0$), the light saturation point (irradiance at 95% of $P_{\text{max}}$), 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 palubara), 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 ($\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$) | Light saturation point ($\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$) | Light respiration point ($\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$) | Dark efficiency rate ($\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$) | Quantum |
|-----------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|----------|
| **Kale**              |                                  |                                 |                                 |                                  |          |
| ‘Red Russian’         | 20.3 ± 0.7 a                    | 13 ± 1.9 a                      | 1.03 ± 2.9                     | –1.12 ± 0.12 c                  | 0.07 ± 0.02 |
| ‘Toscana’             | 18.2 ± 0.3 b                    | 15 ± 2.2 ab                     | 1.04 ± 2                       | –0.97 ± 0.11 c                  | 0.06 ± 0.04 |
| ‘Winterbor’           | 17.4 ± 0.7 ab                   | 6 ± 1 a                         | 905 ± 61                       | –0.39 ± 0.10 c                  | 0.06 ± 0004 |
| **Spinach**           |                                  |                                 |                                 |                                  |          |
| ‘Melody’              | 23.8 C                          |                                 |                                 |                                  |          |
| ‘Harmony’             | 22.4 ± 0.12 d                   | 79 ± 3 c                        | 1.177 ± 102                    | –5.51 ± 0.43 a                  | 0.08 ± 006 |
| ‘Bloomsdale LS’       | 22.0 ± 0.5 e                    | 72 ± 4 e                        | 1.270 ± 16                     | –4.46 ± 0.23 a                  | 0.07 ± 0007 |
| **Swiss chard**       |                                  |                                 |                                 |                                  |          |
| ‘Bright Lights’       | 18.2 A                          | 25 ± 4 b                        | 1.238 B                        | –5.00 A                         | 0.08 B    |
| ‘Fordhook Giant’      | 21.9 ± 0.7 cd                   | 22 ± 2 b                        | 1.021 ± 17                     | –1.49 ± 0.11 bc                 | 0.07 ± 002 |
| ‘Rhubarb’             | 18.0 ± 0.8 abc                  | 41 ± 4 c                        | 939 ± 65                       | –2.60 ± 0.20 b                  | 0.07 ± 004 |
| ‘Yellow’              | 16.3 ± 0.9 a                    | 21 ± 4 b                        | 790 ± 65                       | –1.43 ± 0.31 bc                 | 0.07 ± 004 |

Analysis of Variance

| Species   | *** | *** | *** | *** | * |
|-----------|-----|-----|-----|-----|---|
| Variety   | *** | *** | *** | *** | NS |

| *** | *** | *** | *** | NS |

HSD = honestly significant difference; NS = nonsignificant.

‘Winterbor’ kale had the lowest $P_{\text{max}}$ (14.7 $\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$), and ‘Red Russian’ and ‘Toscana’ kale had the highest $P_{\text{max}}$ (17.8 and 19.0 $\mu$mol CO$_2$·m$^{-2}$·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 $\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$), chard $R_D$ was –1.64 $\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$, and spinach $R_D$ was the highest (–5.00 $\mu$mol CO$_2$·m$^{-2}$·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 $\mu$mol CO$_2$·m$^{-2}$·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 mmol·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 the acclimation of $P_n$ to high 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 Sitch, 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 reduces in $P_n$ rates lower than the $P_{\text{max}}$ 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 $I = I_o e^{-kL}$ [$I = $ irradiance below the canopy; $I_o = $ 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 $\mu$mol·m$^{-2}$·s$^{-1}$) irradiance levels than spinach (1000–1200 $\mu$mol·m$^{-2}$·s$^{-1}$; 400 ppm CO$_2$; Fig. 1; Table 2). $P_{\text{max}}$ 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 (CO$_2$) 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 ± mean square error as identified through analysis of variance ($\alpha < 0.05$).
Table 3. Variation in the predicted maximum photosynthetic rate (P\textsubscript{max}; μmol CO\textsubscript{2}·m\textsuperscript{-2}·s\textsuperscript{-1}), the CCP (CO\textsubscript{2} concentration when P\textsubscript{a} = 0), and the CSP (the CO\textsubscript{2} concentration at 95% P\textsubscript{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\textsubscript{0.05}] across species, and small letters mean separation across cultivars.

| Plant species/cultivar | P\textsubscript{max} (μmol CO\textsubscript{2}·m\textsuperscript{-2}·s\textsuperscript{-1}) | CCP (ppm) | CSP (ppm) |
|------------------------|---------------------------------|-----------|-----------|
| **Kale**               |                                 |           |           |
| ‘Red Russian’          | 17.8 ± 0.5 bcd                  | 58.8 ± 1.5 abc | 858 ± 51 a |
| ‘Toscano’              | 19.0 ± 0.3 cde                  | 64.4 ± 2.1 bcd | 1,013 ± 51 ab |
| ‘Winterboor’           | 14.7 ± 0.5 a                    | 71.8 ± 3.1 d  | 1,170 ± 101 ab |
| Spinach                |                                 |           |           |
| ‘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 |
| ‘Bloomdale LS’         | 20.0 ± 0.4 c                    | 58.0 ± 1.1 abc | 1,057 ± 11 ab |
| **Swiss chard**        |                                 |           |           |
| ‘Bright Lights’        | 17.6 ± 0.6 cde                  | 67.3 ± 3.7 cd | 1,266 ± 124 b |
| ‘Fordhook Giant’       | 17.5 ± 0.7 bcd                  | 64.1 ± 3.1 abcd | 1,113 ± 125 ab |
| ‘Rhubarb’              | 16.9 ± 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** |                                 | ***        | NS        |
| Species                |                                 | ***        | ***        |
| Cultivar               |                                 | **         | **         |

P\textsubscript{max} = maximum photosynthetic rate; CCP = CO\textsubscript{2} compensation point; CSP = CO\textsubscript{2} saturation point; hsd = honestly significant difference; ns = nonsignificant.

| Denotes significance at the α < 0.05 (*), < 0.01 (**), < 0.001 (***). levels.

Based on the instantaneous P\textsubscript{a} data here, kale and chard may be better suited for production in naturally low irradiance locations or facilities than spinach as their LSPs are lower than that of spinach (Table 2). Also, as spinach had a higher LCP (60–75 μmol·m\textsuperscript{-2}·s\textsuperscript{-1}) than kale (10–15 μmol·m\textsuperscript{-2}·s\textsuperscript{-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\textsubscript{a} when irradiance was increased from 300 to 350 μmol·m\textsuperscript{-2}·s\textsuperscript{-1} on kale, chard, and spinach (DLI = +3.24 mol·m\textsuperscript{-2}·d\textsuperscript{-1} for chard and kale, and ±1.44 mol·m\textsuperscript{-2}·d\textsuperscript{-1} for spinach as photoperiod differed). Increasing irradiance from 300 to 350 μmol·m\textsuperscript{-2}·s\textsuperscript{-1} increased predicted spinach P\textsubscript{a} by 15% and that of kale and chard by 9% to 11% (Table 4).

Responses to CO\textsubscript{2}. Kale and chard CSP was lower (600–800 ppm) than that of spinach (1000–1200 ppm) (irradiance = 300 μmol·m\textsuperscript{-2}·s\textsuperscript{-1}; Fig. 1; Table 3). Responses observed on kale here were similar to those observed by others (700 ppm CO\textsubscript{2} 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 CO\textsubscript{2} levels, supplementing greenhouses or growth rooms with CO\textsubscript{2} (above ambient) would likely increase the P\textsubscript{a} of crops studied here (Table 3). In fact, increasing CO\textsubscript{2} to increase CO\textsubscript{2} levels to 800–1000 ppm is commonplace in commercial vegetable production greenhouses to increase yield (personal observation; Dorais, 2003). For instance, increasing CO\textsubscript{2} from 330 to 900 ppm increased tomato yield by 21% (Dorais, 2003).

P\textsubscript{a} in unventilated greenhouses or enclosed rooms can be limited by declining CO\textsubscript{2} levels as plants use CO\textsubscript{2} for photosynthesis. It is not uncommon for CO\textsubscript{2} levels in unventilated greenhouses or enclosed rooms to be limited by declining CO\textsubscript{2} levels as plants use CO\textsubscript{2} for photosynthesis. It is not uncommon for CO\textsubscript{2} levels in unventilated greenhouses or enclosed rooms to be limited by declining CO\textsubscript{2} levels as plants use CO\textsubscript{2} for photosynthesis.

Respiration. Spinach, kale, and chard predicted R\textsubscript{a} (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\textsubscript{a} among species and among varieties of some species. Although spinach variety R\textsubscript{a} was high and similar, kale and chard variety R\textsubscript{a} varied with some varieties having a 4-fold higher R\textsubscript{a} than with other varieties (Table 2). Variation in R\textsubscript{a} among varieties of other vegetable species (such as asparagus) has also been observed (Kitazawa et al., 2011). Such variation in R\textsubscript{a} among species and varieties here is especially important to quantify as R\textsubscript{a} can be negatively correlated with postharvest performance (Kitazawa et al., 2011). For
instance, data here suggest that spinach may have a shorter postharvest life than kales and that among kales varieties and among chard varieties, ‘Bright Lights’ may have a shorter postharvest life than ‘Rhubarb’ (Table 2).

Irradiance versus CO2. The question arises as to whether a spinach, kale, or chard producer should increase irradiance or CO2 to increase yield most. Low CO2 conditions (200 ppm) reduced predicted \( P_n \) more than low irradiance (200 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)) conditions on both kales and chards here (Table 4). Therefore, increasing CO2 levels are at ambient levels (400 ppm) may increase \( P_n \) and likely yield on kales and chards 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 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)) to the LSP increased predicted \( P_n \) more than increasing CO2 from ambient (400 ppm) to the CSP (Table 4). This suggests that irradiance may be more limiting than CO2 with the crops studied here.

Future work must examine the synergy between irradiance and CO2 on kales, chards, and spinach. Increasing CO2 from 400 to 800 ppm and increasing irradiance from 400 to 800 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \) significantly increased lettuce dry weight by 25% (1.5 g/plant) and 19% (1.15 g/plant), respectively (Chagvardieff et al., 1994). However, increasing CO2 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 CO2 suggesting different genetic backgrounds. There was little variation among spinach varieties in \( P_{\text{max}} \), LCP, CCP, 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 \( P_{\text{max}} \) amongst lettuce varieties was observed by Behr and Wiebe (1992). Gu et al. (2012) observed variation in \( P_{\text{max}} \) (17% to 25% variation) in rice (13 lines; *Oryza sativa* L.) at ambient CO2 levels (380 ppm) and that variation was associated with stomatal and mesophyll conductance. Yu et al. (2016) found variation in *Cucumis* *pumilus* 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 \( g_s \) (Kaizer 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 \( P_n \) (Rd already quantified in direct \( P_n \) readings) and \( R_D \) (at night only) into account [net carbon gain = \([P_{\text{max}} \times 18 \text{ h}\text{d}^{-1} \text{ (kale)} \text{ or } 8 \text{ h}\text{d}^{-1} \text{ (spinach)}] - \{R_D \times 8 \text{ h}\text{d}^{-1} \text{ (kale)} \text{ or } 16 \text{ h}\text{d}^{-1} \text{ (spinach)}\} \), we observed spinach carbon gain/d (380.8–80.0 = 300.8 \( \mu \text{mol} \text{CO}_2 \text{m}^{-2} \text{s}^{-1} \)) was lower than that of kale (365.4–6.6 = 358.8 \( \mu \text{mol} \text{CO}_2 \text{m}^{-2} \text{s}^{-1} \)) even though \( P_{\text{max}} \) was greater (Table 2).

Take home messages.

1. Kale and chard \( P_{\text{max}} \) saturated at lower (600–800 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)) irradiance levels than spinach \( P_{\text{max}} \) (1000–1200 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)); 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 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)) than kale (10–15 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)), 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 CO2 levels, supplementing CO2 would increase the \( P_n \) of crops studied here.

3. Ventilating greenhouses to increase CO2 from 200 to 400 ppm may result in a greater increase in \( P_n \) than that from increasing CO2 from 400 to 800 ppm.

4. Although spinach variety \( R_D \) was similar, kale and chard \( R_D \) varied with some varieties having a 4-fold higher \( R_D \) than others.

5. Low CO2 reduced kale and chard \( P_n \) more than low irradiance. Therefore, ventilating production spaces to ensure CO2 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 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \) to the CSP increased predicted \( P_n \) and \( R_D \) that is more limiting than CO2 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 \( P_{\text{max}} \) was greater.

**Literature Cited**

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