Omega-3 Fatty Acid Concentration in Purslane (Portulaca oleracea) is Altered by Photosynthetic Photon Flux

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Abstract. Purslane (Portulaca oleracea L.) is an excellent source of the essential fatty acid α-linolenic acid (LNA) but little is known of the effects of cultural conditions on LNA concentration. Purslane seedlings were grown under an instantaneous photosynthetic photon flux (PPF [400 to 700 nm]) of 299 or 455 μmol·m⁻²·s⁻¹ for a daily duration of either 8, 12, 16, or 20 hours. Thus, plants were exposed to a daily PPF of 8.6, 12.9, 17.2, or 21.5 mol·m⁻²·d⁻¹ in the low PPF treatment (299 μmol·m⁻²·s⁻¹) and 13.1, 19.7, 26.2, or 32.8 mol·m⁻²·d⁻¹ in the high PPF treatment (455 μmol·m⁻²·s⁻¹). Plants in all treatments received a 20-hour photoperiod by providing ≈5 μmol·m⁻²·s⁻¹ from incandescent lamps starting at the end of the photosynthetic light period. At low PPF, purslane grown under a 16-hour PPF duration produced the highest concentrations of total fatty acids (TFA) and LNA per unit leaf dry weight (DW), but at high PPF, concentrations of these compounds were highest under 8 and 12 hour PPF duration. Trend analysis indicated that maximum TFA and LNA concentrations occurred with a daily PPF of 14.1 and 17.2 mol·m⁻²·d⁻¹, respectively; and in the thylakoids, protein, chlorophyll, and LNA concentrations peaked at a PPF of 21.8, 19.9, and 16.1 mol·m⁻²·d⁻¹, respectively. LNA as a percentage of TFA was unaffected by treatment. Shoot DW increased with PPF up to the highest PPF exposure of 32.8 mol·m⁻²·d⁻¹.

Materials and Methods

Plant Material. Fourteen-day-old purslane seedlings (Valley Seed Service, Fresno, Calif.) were transplanted into 500-cm³ square pots containing the commercial medium Metro 510 (O.M. Scotts, Marysville, Ohio), a mixture of sphagnum peat, vermiculite, and composted pine bark. Thirty-two pots were transferred to each of four growth chambers (model G-10; Environmental Growth Chambers, Chagrin Falls, Ohio), equipped with cool-white fluorescent/incandescent light, programmed to maintain 12-h days/12-h nights of 27/22 °C.

Plant Growth Environment. Within each chamber, plants were arranged at two levels, so that 16 plants received an average instantaneous PPF (400 to 700 nm) of 455 μmol·m⁻²·s⁻¹, and the other 16 plants received an average PPF of 299 μmol·m⁻²·s⁻¹. Plants were grown under a PPF of 299 or 455 μmol·m⁻²·s⁻¹ for a daily duration of either 8, 12, 16, or 20 h. Total cumulative PPF received by the plants per day was 8.6, 12.9, 17.2, or 21.5 mol·m⁻² for low PPF (299 μmol·m⁻²·s⁻¹), and 13.1, 19.7, 26.2, or 32.8 mol·m⁻² for high PPF (455 μmol·m⁻²·s⁻¹). The daily photoperiod was maintained at 20 h for all treatments by providing ≈5 μmol·m⁻²·s⁻¹ from incandescent lamps starting at the end of the photosynthetic light

Recent interest in the dietary intake of omega-3 fatty acids (ω3FA) has been stimulated by a greater understanding of their role in normal human growth, development, and disease prevention (Simopoulos, 1991, 1999; Simopoulos et al., 1986). α-Linolenic acid (LNA) is an ω3FA that is essential in the human diet as a precursor for the synthesis of longer chain fatty acids and the prostaglandin group of mammalian hormones (British Nutrition Foundation, 1993).

Purslane (Portulaca oleracea) is an excellent dietary source of both LNA and the antioxidant, α-tocopherol (Simopoulos et al., 1992), and because of this, there is renewed interest in the cultivation of purslane as a food crop (Adams, 1992; Levey, 1993). Although purslane has been studied in detail as a prolific weed (Vengris et al., 1993), very little is known about the effects of cultural conditions on its nutritional value (LNA content).

Lipid content and lipid metabolism in plants can be altered by a number of environmental factors including light and temperature (Harwood, 1995), mineral nutrient composition of the growing medium (Palaniswamy et al., 2000), and crop maturity (Hudson and Karis, 1974). Omara-Alwala et al. (1991) reported significant differences in the levels of total fatty acids (TFA) and ω3FA in leaves and stems of purslane harvested at three different ages. Although these studies identified a few of the key environmental factors that may influence LNA concentrations in purslane leaves, many environmental conditions were not carefully controlled or monitored.

Several reports in the literature stress a close association between LNA and chlorophyll synthesis, suggesting a close positive relationship between LNA and chlorophyll synthesis. For instance, increased photosynthetic photon flux (PPF) induced a correspond-
period. Irradiance was measured at terminal leaf height using a quantum sensor (LI-190SA; LI-COR, Inc., Lincoln, Nebr.).

The PPF in each growth chamber was greatest in the center row of the chamber and lower along the sides of the chamber. The gradient in the 299 µmol·m⁻²·s⁻¹ treatments ranged from 318 µmol·m⁻²·s⁻¹ along the center of the chamber to 272 µmol·m⁻²·s⁻¹ along the sides of the chamber. The gradient in the 455 µmol·m⁻²·s⁻¹ treatments ranged from 463 µmol·m⁻²·s⁻¹ along the center row to 441 µmol·m⁻²·s⁻¹ along the rear end of the chamber. To insure that all plants received similar PPF during the study, the plants in each treatment were moved every day incrementally from the center of the chamber to the edges of the chamber in a continuous cycle across the light gradient. It took 16 d to complete one rotation. The PPF values reported in this study were obtained by averaging the light measurements taken at various locations in the growth chamber every 5 d throughout the study period.

Treatments were arranged in a split-plot design with PPF duration as the main plot and instantaneous PPF as the subplot. The experiment was replicated in triplicate over time. Treatments were randomly assigned to chambers, resulting in a different chamber being used for each treatment each time the study was replicated. Plants were fertilized with N at 50 µg·mL⁻¹ for the first week and then twice weekly with N at 100 µg·mL⁻¹ until harvest using a 20N–4.36P–16.6K water-soluble fertilizer (The Scotts Co., Marysville, Ohio).

As plants reached the 14-true-leaf stage, four plants were harvested from each treatment group and shoot fresh weight (FW), dry weight (DW), leaf area, and the number of lateral shoots arising from the main stem were determined. The shoot DW was determined after drying at 60°C for 24 h. Leaf area was measured with a leaf area meter (LI 3100; LI-COR). At the same time, an additional eight plants were selected at random, and young, fully expanded leaves (leaves from the third, fourth and fifth nodes from the shoot tip), were harvested for chemical analysis. The harvested sample was divided into two subsamples. One subsample was used to determine thylakoid chlorophyll, fatty acids, and protein concentrations, and the other subsample was used to determine whole leaf chlorophyll concentration, and whole leaf fatty acid composition and concentrations.

**THYLAKOID RECOVERY.** Thylakoid membranes were recovered from leaf samples by grinding =4 g of leaf tissue in 20 mL of a 50 mM phosphate buffer (pH 7.6) containing 0.4 M sorbitol, 20 mM NaCl, 20 mM HEPES, and 3 mM MgCl₂·6H₂O (Berkowitz and Gibbs, 1985; Chapman et al., 1983). The filtrate was centrifuged at 3000 gₛ for 4 min, and the pellet was washed in 20 mL of a 50 mM phosphate buffer (pH 7.6) containing 20 mM NaCl and shaken on ice for 15 min. The thylakoid pellet was recovered following centrifugation at 10,000 gₛ for 10 min.

**CHLOROPHYLL ANALYSIS.** Thylakoid chlorophyll concentration was determined by suspending a 50-µL sample of thylakoid membranes in 4 mL of 80% acetone and recording the absorbance at 645 nm and 663 nm (Arnon, 1949). Whole leaf chlorophyll was determined by extracting chlorophyll from leaf discs with N,N-dimethyl formamide and measuring absorption values at 647 nm and 664 nm (Inskipp and Bloom, 1985). The ratio of whole leaf chlorophyll to thylakoid chlorophyll was used to determine the percentage thylakoid recovery for each sample. The recovery factor was also used to estimate thylakoid protein on a leaf DW basis.

**PROTEIN ANALYSIS.** Protein concentration was determined using the spectrophotometric procedure of Lowry et al. (1951). A 50-µL sample of thylakoid was suspended in 150 µL of 0.85% NaCl and 2.2 mL of Biuret reagent was added. After 30 min, 100 µL of phenol reagent was added, mixed well, and the absorbance at 750 nm was recorded. Thylakoid protein was expressed as mg·g⁻¹ leaf DW.

**FATTY ACID EXTRACTION, PREPARATION AND ANALYSES.** Lipids were extracted using the dry column method (Maxwell et al., 1980). Leaf tissue (=4 g) was homogenized for 30 s in 25 mL of 9 dichloromethane (DCM) : 1 methanol (v/v). The homogenate was mixed with 4 g anhydrous sodium sulfate and then flushed through a glass column [22 mm x 30 cm packed with 1 calcium phosphate : 9 celite (w/w)] with 150 mL DCM: methanol (9:1, v/v). The filtrate was collected and vacuum dried in a rotary evaporator. The dried lipid fraction was dissolved in 1 mL DCM and transferred to 2-mL glass screw-cap vials, flushed with nitrogen, and stored under refrigeration until transesterification.

The transesterification procedure of Lepage and Roy (1986) was used to methylate the fatty acid fraction. A 100-µL aliquot of extracted lipid was mixed with 2 mL of an internal standard [100 µL·mL⁻¹ of heptadecanoic acid (a 17:0 fatty acid) in 4 methanol : 1 hexane (v/v)] and 200 µL acetic acid in a screw cap test tube. Test tubes were sealed with Teflon thread seal tape and heated for 1 h at 100°C. After cooling, 5 mL of 6% K₂CO₃, was added slowly, mixed, and then centrifuged at 5000 gₛ for 10 min. The lipid layer was removed, dried completely under nitrogen gas, and then dissolved in 50 µL DCM. Finally, the resulting fatty acid methyl esters were injected into a gas chromatograph (Varian 6000, Palo Alto, Calif.) for fatty acid separation and analysis. Fatty acid peak areas were determined with a Hewlett Packard 3395 integrator (Wilmington, Del.) and identified by comparing retention times with standards separated under similar chromatographic conditions [poly unsaturated fatty acid (PUFA-2), Matreya, Inc., Pleasant Gap, Pa.].

**CHROMATOGRAPHIC CONDITIONS.** The gas chromatograph was fitted with Supelcowax capillary column [10 stationary phase, 1 µm film thickness, 0.53 mm i.d. x 30 m in length (Supelco, Bellefonte, Pa.) and a flame ionization detector. Air and hydrogen flow rates at the detector were maintained at 300 and 30 mL·min⁻¹, respectively, and the nitrogen carrier gas flow rate was 1 mL·min⁻¹. Column temperature gradient increased from 190 to 235°C at a rate of 2°C·min⁻¹ and ion temperature was held at 240°C. Analysis time was =30 min/sample.

**DATA ANALYSIS.** Data were analyzed using SAS General Linear Models procedures (SAS Inst. Inc., 1994). Orthogonal polynomial contrasts were performed for significant responses and regression equations were generated.

Trendlines were fitted using Microsoft Power Point (7.0) software package and the Xmax for the significant responses (Y) were calculated as Xmax= –b/2c, where X is the treatment, and b and c are the regression coefficients in the normal equation: Y =a+bx+cX².

**TRANSMISSION ELECTRON MICROSCOPY.** Transverse sections of purslane leaves were prepared and examined under the electron microscope (Sims and Pearcy, 1992). Small pieces (=1 mm) of fully expanded leaves were put in a fixative containing 8% glutaraldehyde and 10% formaldehyde and fixed by rotation on a rotating platform for =8 h. The sections were then washed three times with distilled water and later three more times with 0.1 M phosphate buffer to remove the fixatives. The samples were postfixed in osmium with 1% osmium in 0.1 M phosphate buffer, rotated for 2 h to remove the osmium and washed twice with distilled water. The sections were dehydrated in a graded ethanol series (30%, 50%, 70%, 95%, and 100%) and then twice in propylene oxide each at 0.5-h intervals. The dehydrated sections were fixed in resin (propylene oxide and resin, 1:1 ratio), left at =22°C for =8 h, and later embedded in the resin mix and dried in the oven at 60°C for 24 to 36 h in a
Thin sections were cut from the embedded leaf sections and mounted on copper grids [200 mesh (0.127 mm)] stained with lead citrate and examined under a transmission electron microscope (EM 300; Philips, Eindhoven, The Netherlands) at the University of Connecticut Electron Microscopy Laboratory.

Results

Relative concentration of individual fatty acid species, as a percentage by weight of TFA in purslane leaves were not influenced by total daily PPF. Mean fatty acid composition (all expressed as percentage of TFA ± se) averaged across all light treatments was 16.3 ± 1.0 for palmitic (16:0), 2.7 ± 0.2 for palmitoleic (16:1), 1.2 ± 0.1 for stearic (18:0), 3.9 ± 0.4 for oleic (18:1), 13 ± 0.6 for linoleic (18:2), and 62.9 ± 2.2 for linolenic (18:3).

Regression analysis showed that TFA (Y = –0.0645x² + 1.8237x + 23.457; r² = 0.54) and LNA (Y = –0.0643x² + 2.2168x + 2.8427; r² = 0.54) concentrations in purslane leaves peaked at a cumulative PPF of 14.1, and 17.2 mol·m⁻²·d⁻¹ respectively. However, maximum FW was recorded in plants receiving a PPF of 26.4 mol·m⁻²·d⁻¹ (Fig. 1) and thylakoid protein, chlorophyll, and LNA concentrations were maximized at cumulative PPF of 21.8, 19.9, and 16.1 mol·m⁻²·d⁻¹ respectively (calculated from data in Table 1).

At low irradiance (299 µmol·m⁻²·s⁻¹), TFA and LNA concentrations in purslane leaves were 81% and 79% higher, respectively, at 16 h PPF duration (17.2 mol·m⁻²·d⁻¹) than at 20 h PPF (21.5 mol·m⁻²·d⁻¹) (Table 1). However, at high irradiance (455 µmol·m⁻²·s⁻¹), the concentrations of TFA and LNA per unit leaf DW decreased as PPF duration increased from 12 to 20 h. Similar concentrations of TFA and LNA (per unit leaf DW) were found in the leaves of purslane grown under 8 and 12 h PPF duration (13.1 and 19.7 mol·m⁻²·d⁻¹). Under a 16 h PPF duration, TFA and LNA concentrations in purslane grown under the low irradiance were 124% and 136% higher, respectively, than in purslane grown under the high PPF (Table 1).

LNA concentrations in the thylakoid fraction recovered from plants in the various PPF treatments followed the same pattern as observed for whole leaf TFA and LNA (Table 1). At low PPF, thylakoid LNA concentration increased as PPF duration increased from 8 to 16 h and then decreased dramatically at 20 h. At high PPF, thylakoid LNA concentration declined steadily as PPF duration increased from 8 to 20 h.

Thylakoid protein concentrations followed a different pattern than thylakoid LNA in response to the various PPF treatments (Table 1). At low PPF, thylakoid protein concentrations increased steadily as PPF duration increased, from 261 mg·g⁻¹ leaf DW at 8

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**Table 1. Effect of total daily photosynthetic photon flux (PPF, mol·m⁻²·d⁻¹) on total fatty acids (TFA) and α-linolenic acid (LNA) concentrations in leaves of purslane. Data represent means of three observations.**

| PPF treatment | Instantaneous Avg total | Whole-leaf concn | Thylakoid concn |
|---------------|------------------------|-----------------|-----------------|
|               | PPF (µmol·m⁻²·s⁻¹)     | PPF (mol·m⁻²·d⁻¹) | TFA (mg·g⁻¹ leaf DW) | LNA (mg·g⁻¹ leaf DW) | Protein | Chlorophyll |
| 8 h           | 299                    | 8.6             | 33.2            | 15.1            | 2.2      | 261        | 16.6        |
|               | 455                    | 13.1            | 33.7            | 21.7            | 10.6     | 303        | 16.2        |
| 12 h          | 299                    | 12.9            | 34.0            | 19.2            | 11.8     | 295        | 20.3        |
|               | 455                    | 19.7            | 37.6            | 23.9            | 5.6      | 471        | 20.7        |
| 16 h          | 299                    | 17.2            | 50.0            | 32.3            | 12.1     | 344        | 21.7        |
|               | 455                    | 26.2            | 22.3            | 13.7            | 3.5      | 532        | 21.9        |
| 20 h          | 299                    | 21.5            | 23.1            | 15.2            | 3.5      | 555        | 21.4        |
|               | 455                    | 32.5            | 16.8            | 10.6            | 1.5      | 217        | 12.5        |

| Source of variation | df | F value | F value | F value | F value |
|---------------------|----|---------|---------|---------|---------|
| PPF duration (h)    | 3  | 3.9*    | 29.9*   | 18.6    | 7.6*    | 6.9*    |
| PPF (irradiance)    | 1  | 9.4*    | 11.1*   | 3.6ns   | 0.6ns   | 3.9ns   |
| Total PPF (duration × irradiance) | 3  | 8.4*    | 22.3*   | 50.5**  | 14.8**  | 4.0**ns |
| LSD₀.₀₅             | 13.1| 10.0    | 6.9     | 226.0   | 4.2     |

*ns*** Nonsignificant or significant at P ≤ 0.05, 0.01, or 0.001 respectively.
Table 2. Effect of photosynthetic photon flux (PPF) on the relative ratio of thylakoid protein, chlorophyll, and α-linolenic acid (LNA) and the ratio of thylakoid to whole leaf LNA in purslane. Data represent means of three observations.

| PPF treatment | Instantaneous PPF (µmol·m⁻²·s⁻¹) | Avg total PPF (mol·m⁻²·d⁻¹) | Ratios in thylakoid | Ratio of thylakoid LNA to total LNA |
|---------------|----------------------------------|-----------------------------|---------------------|-----------------------------------|
|               |                                  |                             | LNA: protein        | LNA: chlorophyll                  | Protein: chlorophyll               |                                           |
| 8 h           | 299                              | 8.6                         | 8.5                 | 132                               | 15.8                             | 146                                       |
| 12 h          | 299                              | 12.9                        | 12.6                | 586                               | 14.6                             | 615                                       |
|               | 455                              | 19.7                        | 38.1                | 551                               | 15.5                             | 375                                       |
| 16 h          | 299                              | 17.2                        | 6.8                 | 162                               | 24.6                             | 255                                       |
|               | 455                              | 26.2                        | 6.9                 | 162                               | 25.7                             | 230                                       |
| 20 h          | 299                              | 21.5                        | 6.8                 | 116                               | 16.9                             | 142                                       |
|               | 455                              | 32.5                        |                      |                                   |                                  |                                           |

Source of variation | df | F value | F value | F value | F value |
|---------------------|----|---------|---------|---------|---------|
| PPF duration (h)    | 3  | 27.3*** | 18.6*   | 14.5**  | 7.7**   |
| PPF (irradiance)    | 1  | 10.7*   | 3.6**   | 4.5**   | 2.4**   |
| Total PPF (duration × irradiance) | 3  | 31.8*** | 50.5*** | 9.5**   | 11.0**  |
| LSD₀.₀⁵             | 25.8 | 385.0   | 7.4     | 206.0   |

Nonsignificant or significant at P ≤ 0.05, 0.01, or 0.001 respectively.

h to 555 mg·g⁻¹ leaf DW at 20 h. At high PPF, thylakoid protein concentrations increased as PPF duration increased from 8 to 16 h, and then declined dramatically at 20 h.

Thylakoid LNA concentration per unit thylakoid protein was affected by total PPF (Table 2). At low PPF irradiance, the ratio of thylakoid LNA to protein increased over 4.5-fold when PPF duration increased from 8 h to 12 h or 16 h. At 20 h PPF duration, thylakoid LNA to protein ratio was similar to that observed at 8 h. However, at high PPF irradiance the thylakoid LNA to protein ratio decreased over 65% when PPF duration increased from 8 h to 12 h, and decreased an additional 46% when PPF duration increased from 12 h to either 16 h or 20 h. Changes in the thylakoid LNA to thylakoid chlorophyll ratio in response to the various PPF treatments were similar to those observed for the thylakoid LNA to protein ratio (Table 2).

In contrast, the ratio of thylakoid protein to thylakoid chlorophyll in leaves exposed to low PPF irradiance was unchanged as PPF duration increased from 8 to 16 h, and then increased at 20 h PPF duration (Table 2). The ratio of thylakoid protein to thylakoid chlorophyll in purslane leaves exposed to the high PPF irradiance increased gradually as the PPF duration increased from 8 to 16 h, and then decreased at 20 h PPF duration. Thylakoid chlorophyll concentration was lowest at the average PPF of 32.5 mol·m⁻²·d⁻¹ (Table 1).

LNA recovered in the thylakoid fraction relative to the total LNA in the whole leaf varied dramatically with PPF treatment (Table 2). At the low PPF irradiance, LNA recovered in the thylakoid fraction increased from 14.6% of the total whole leaf LNA at the 8 h PPF duration to 61.5% of the total at the 12 h PPF duration, and then declined steadily to 23% at the 20 h PPF duration. At the high PPF irradiance, LNA in the thylakoid accounted for over 48% of the total whole leaf LNA when plants were grown at an 8 h PPF duration, but then declined at longer
Table 3. Effect of total daily photosynthetic photon flux (PPF, mol·m⁻²·d⁻¹) on growth of purslane. Data represent means of three observations.

| PPF treatment | Instantaneous PPF | Avg total PPF | Growth parameter |
|---------------|-------------------|--------------|------------------|
|               | (µmol·m⁻²·s⁻¹)    | (mol·m⁻²·d⁻¹) | Leaf DW (g) | Shoot DW (g) | Leaf area (cm²) | Shoot no. | Plant ht (cm) |
| PPF duration (h) |                   |             |              |              |              |          |              |
| 8             | 299               | 8.6         | 0.11         | 0.3          | 72            | 0.9        | 24.7         |
|               | 455               | 13.1        | 0.39         | 0.9          | 145           | 2.5        | 31.5         |
| 12            | 299               | 12.9        | 0.46         | 1.1          | 141           | 2.5        | 30.1         |
|               | 455               | 19.7        | 0.69         | 1.6          | 263           | 3.0        | 35.7         |
| 16            | 299               | 17.2        | 0.54         | 1.3          | 213           | 3.1        | 36.6         |
|               | 455               | 26.2        | 0.86         | 1.7          | 293           | 3.3        | 34.8         |
| 20            | 299               | 21.5        | 0.64         | 1.3          | 228           | 3.3        | 27.0         |
|               | 455               | 32.5        | 1.0          | 1.9          | 270           | 3.1        | 27.8         |

Source of variation df F value F value F value F value F value
PPF duration (h) 3 111.6** 106.2** 26.8*** 166.1*** 17.7**
PPF (irradiance) 1 79.9*** 37.8*** 30.1*** 29.6*** 10.7**
Total PPF (h × irradiance) 3 0.9ns 0.2ns 1.3ns 16.2** 5.5*
LSD 0.05 6.2 0.3 50.2 0.7 3.8

NS, **, *** Nonsignificant or significant at P ≤ 0.05, 0.01, or 0.001 respectively.

PPF duration to a low of 14.2% in purslane grown under a 20 h PPF duration. Micrographic analysis of purslane leaf sections revealed the presence of numerous osmiophilic lipid globules inside the large chloroplasts (Fig. 2).

While the relationship between total PPF and TFA concentration is complex (Fig. 1), the relationship between plant growth and total PPF is clearer (Table 3). Purslane shoot DW, leaf DW, and leaf area all increased with increased total PPF (Table 3). At both low and high PPF irradiance, plant growth (as represented by leaf DW, shoot DW, and total leaf area) increased with increased PPF duration.

Plant growth habit was influenced greatly by the interplay between PPF duration and irradiance. The number of lateral shoots that developed on each plant increased =2.5 to 3.5-fold when PPF duration increased from 8 h to 12 or more hours at the low PPF irradiance (Table 3). However, at the high PPF irradiance the number of lateral shoots varied little with PPF duration from a minimum of 2.5 shoots/plant under the 8 h PPF duration to a maximum of 3.5/plant under the 16 h PPF duration. At the low PPF irradiance, plant height increased with increased PPF duration up to 16 h, but then decreased at 20 h PPF duration. At the high PPF irradiance, plant height increased only slightly as PPF duration increased from 8 h to 12 h and then decreased at the 20 h PPF duration.

Discussion

Total PPF exerted a strong influence over fatty acid concentrations in purslane leaves, but did not alter LNA as a percentage of TFA. Though TFA and LNA concentrations were altered by the PPF treatments, LNA as a percentage of TFA remained the same in all the treatments (Table 1). Burkey et al. (1997) reported that in soybean [Glycine max (L.) Merrill] leaves maintained relatively constant ratios of fatty acids under various light exposures.

The relationship between PPF and whole-leaf TFA, and between PPF and thylakoid protein, is complex in that the response to PPF irradiance appears to be dependent on PPF duration. Whole-leaf TFA concentration reached a maximum at a cumulative daily PPF of 14.1 mol·m⁻²·d⁻¹, which was attained after 8.6 h at high PPF and 13.1 h at low PPF and then decreased at higher cumulative PPFs (Fig. 1). A similar trend was also observed for LNA (Table 1). Thus, fatty acid concentrations in purslane leaves were determined by the total daily PPF exposure rather than by irradiance or duration.

In contrast, chlorophyll concentration increased linearly with increased PPF duration regardless of PPF irradiance (Table 3). Chlorophyll concentration increased as PPF increased to 20 mol·m⁻²·d⁻¹, and then decreased (Table 1). Total LNA concentration peaked at a lower PPF than did chlorophyll and then declined at a more rapid rate than chlorophyll at high daily PPF.

Thylakoid protein, chlorophyll, and LNA concentrations are influenced by cumulative daily PPF (mol·m⁻²·d⁻¹) received by the plant, but regression analysis showed they peaked at a different total PPF. Protein and chlorophyll accumulations were maximized at a higher total PPF (21.8 and 19.9 mol·m⁻²·d⁻¹, respectively) than was thylakoid LNA (16.1 mol·m⁻²·d⁻¹) or total leaf LNA (17.2 mol·m⁻²·d⁻¹). These results indicate that LNA accumulation in purslane was not necessarily linked to chlorophyll (Palaniswamy et al., 2000) or protein concentration as observed earlier in other plants (Melis, 1984; Murphy and Stumpf, 1979; Tchang et al., 1985; Tremolieres, 1972; Tremolieres et al., 1973, 1979).

LNA: chlorophyll in leaves was highest in the thylakoid fraction extracted from plants grown under high PPF for 8 h followed by those grown under low PPF for 12 to 16 h (Table 2). A similar trend was also seen in the LNA: protein ratio in the leaves. Chapman et al. (1983) observed higher lipid to chlorophyll ratio in leaves of pea grown under winter conditions (normally associated with shorter photosynthetic light periods and lower irradiiances). Chapman et al. (1982) hypothesized that chlorophyll levels are a reflection of protein content, but in our study changes in chlorophyll concentration did not coincide directly with changes in protein concentration (Table 1). Unlike pea, purslane is a C₃ plant that exhibits crassulacean acid metabolism under short photoperiod or water stress conditions commonly associated with a high light environment (Koch and Kennedy, 1980).

Though Anderson and Beardall (1991) reported that there is “synchronized synthesis of chlorophyll, LNA and proteins” to enable the correct assembly of lipids and proteins leading to the formation of uniform lamellar bilayers of the thylakoid membranes in plants, our results indicate that synthesis of each of these membrane components in purslane may be influenced differently by PPF.
irradiance and duration, reflecting the different roles these compounds play in plant metabolism.

Barta (1975) observed that lipid synthesis was related positively to the rate of photosynthesis and DW accumulation. With purslane, DW accumulation continued to increase with higher PPF and longer durations, and LNA accumulation did not. Since chlorophyll, protein, and LNA synthesis are influenced differently by the instantaneous PPF, PPF duration, and the cumulative daily PPF, it is evident that LNA synthesis/accumulation in purslane leaves was not directly linked to the DW accumulation as suggested by Barta (1975). Simopoulos et al. (1992) reported that purslane grown in growth chambers at an 18 h photoperiod, with a PPF of 200 µmol·m⁻²·s⁻¹, and days/nights of 24/17°C, were richer in ω3FA than were plants grown in the summer at Beltsville, Md., under field conditions.

In soybean leaves, Burkey et al. (1997) observed a shift in carbon metabolism toward the storage lipids at low irradiance. They suggested that the shift occurred because export of photosynthates from the leaves was either prohibited or the demand for photosynthates by sink tissues was limited. Extra-thylakoidal LNA (the portion of total LNA not associated with the thylakoid membrane) tended to increase as thylakoid LNA decreased and decreased as thylakoid LNA increased. Conditions that reduce photosynthesis such as water stress can cause a decline in carbon export from soybean leaves (Huber et al., 1984), and increase storage lipids in cotton (Gossypium hirsutum L.) leaves (Wilson et al., 1987) and maize (Zea mays L.) leaves (Douglas and Paleg, 1981).

Based on our data one can speculate that purslane plants invest more carbon in the synthesis of chloroplasts under conditions that favor C₃ metabolism rather than crassulacean acid metabolism. In addition, at very high PPF excess light may cause photoinhibition or damage to the photosynthetic apparatus requiring a high percentage of stored fatty acids for repair purposes. Thus, even though TFA and LNA were relatively low under the highest PPF treatments, the percentage of extra-thylakoidal LNA was very high. In contrast, at moderate light levels (≈13 mol·m⁻²·d⁻¹) TFA and LNA concentrations were very high, but the percentage of stored LNA was relatively low.

Most LNA in higher plants is associated with the chloroplasts. In addition LNA is the major constituent of the thylakoid membranes (Tremolieres, et al., 1979)—69% in spinach (Spinacea oleracea L.) (Allen et al., 1966), 65% in wheat (Triticum aestivum L.) (Wolff, 1966), and 72% in broad bean (Vicia faba L.) (Crombie, 1958). However, the percentage of total leaf LNA associated with purslane thylakoid membranes in our study varied dramatically with total daily PPF.

Simopoulos and Salem Jr. (1986) observed that LNA concentration of purslane was about six times higher than in spinach, but chlorophyll concentration was lower than that of spinach [average chlorophyll concentration of spinach was 44.7 mg·cm⁻², and that of purslane was 37.7 mg·cm⁻² (personal observations)].

Results herein indicate that LNA/protein and LNA/chlorophyll ratios were altered by the various treatment conditions without changing the relative proportions of the lipid species (Table 2). Perhaps this provided an adaptive mechanism to maintain the optimal fluidity of the thylakoids, and negating the need for change in the fatty acid saturation levels or the relative proportions of the lipid classes (Miller and Raison, 1980). Our data also indicate that PPF can be manipulated by supplemental lighting in winter and shading during summer to improve the nutritional value (LNA content) of greenhouse-grown purslane.

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