Effect of Irradiance and Temperature on Composition of Spinach

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Abstract. The composition of spinach (*Spinacea oleracea* L.) was studied in response to daily light integral (DLI) and diurnal variation in a greenhouse. Values for plantings with different irradiance were compared using normalized daily light integral (NDLI), which was DLI divided by leaf area index. The dry mass as a ratio of fresh mass increased with NDLI as it increased from 3 to 27 mol·m⁻²·d⁻¹. Reduced nitrogen (N) changed with time of day under high but not under low NDLI. Nitrate and amino acids were affected by temperature more than NDLI. Starch increased with NDLI to 27 mol·m⁻²·d⁻¹ in morning or afternoon. However, sugars decreased with temperature more than with NDLI, due to a decrease in petioles up to 20 °C. Oxalic acid increased with NDLI or temperature. Over a diurnal cycle, starch had minimum value at 0800 HR and maximum at 1800 HR in all parts. The sugars, sucrose, glucose, and fructose, had a binary response with high values in the day and low values in the night. Oxalic acid increased at the end of the day. Other metabolites had no response to time of day. The growth of spinach may be slow in fall compared with summer due to the effect of low temperature on metabolism of sugars and nitrate.

Spinach (*S. oleracea*) a crop that can be grown in any season, tends to accumulate nitrate under low light (Okazaki et al., 2008; Proietti et al., 2004). The electrical conductivity (EC) and the ratio of nitrate to other nutrients decrease the concentration of nitrate in lettuce (*Lactuca sativa* L.) in winter (Gent, 2003). However, it is unclear to what extent the same treatment will reduce nitrate concentrations in spinach.

The rank of nitrate in salad greens including (*Eruca sativa*, *Beta vulgaris*, and *Brassica oleracea*) purchased in the market was arugula > swiss chard > spinach > lettuce > cabbage (Santamaria et al., 1999). For most vegetables, nitrate was related to dry matter. A diurnal pattern occurred for nitrate uptake and reduction in spinach. Diurnal variation of nitrate in spinach was more common than for lettuce in studies of the two crops grown at 46° and 64° N latitudes (Neely et al., 2010). Nitrate in the shoot increased over a 14-h dark period and decreased in the light in a controlled environment (Scaife and Schloemer, 1994). When spinach was grown at low light intensity, the nitrate concentrations were 42 and 13 mmol·kg⁻¹ in leaf blade and 200 and 195 mmol·kg⁻¹ in petiole at dawn and dusk, respectively; there was rapid uptake of nitrate in the first 6 h of night, then it remained constant until dawn (Steingrover et al., 1986a).

Removing nitrate from the solution before harvest is one method to lower nitrate in hydroponic spinach. Transferring spinach to a nitrogen-free solution for 2 or 3 d lowered leaf nitrate and increased ascorbic acid

(Mozafar, 1996). A switch to ammonium in a nutrient film production system decreased growth, nitrate, and oxalate, but increased dry matter content (Elia et al., 1998). Supplemental light at night lowered nitrate by 60% and 9% in the leaf blades and petiole, respectively, compared with a control with no supplemental light (Steingrover et al., 1986b). When nitrate was withdrawn for 2 d after reaching market size, it did not affect spinach fresh mass (Fukuda et al., 1999), but sap nitrate was reduced with supplemental light. After 4 d of nitrate withdrawal, leaf blades and petioles had 600 and 4000 mg·kg⁻¹ (10 and 65 mmol·kg⁻¹) nitrate, respectively (Fukuda et al., 1999). When hydroponic spinach in a controlled environment was switched to no nitrate or low light, growth as measured by dry mass ceased at 4 and 5 d with N withdrawal and 4 and 2 d with low light, in the shoot and root, respectively (Buyse et al., 1996).

Spinach and swiss chard have high oxalate concentrations. Oxalate was greater in blades than in petioles and greater in petioles than in roots (Santamaria et al., 1999). Likewise, comparisons of many cultivars of spinach grown in four seasons in the field found differences in nitrate and oxalate concentrations (Kaminishi et al., 2004; Kaminishi and Kita, 2006). Nitrate content differed among seasons. Nitrate was 11% higher in winter than in other seasons and oxalate was 24% higher in winter. This result suggests oxalate will be high when nitrate is high.

There is little effect on yield or quality of hydroponic spinach produced at root-zone temperatures of 20, 24, or 28 °C in summer or 15, 20, or 25 °C in winter. The best yield was at 20 °C for six of nine cultivars in summer and at 25 °C for six of eight cultivars in winter (Ikeda et al., 1995). This variation was

caused by increased leaf weight in summer and by leaf width and number in winter.

Differences in the metabolite profiles of hydroponic spinach leaves were measured for different concentrations of nitrate in solution. Soluble amino acids were 5% to 12% of total nitrogen in spinach with free glutamine predominant (Eppendorfer and Bille, 1996). When spinach was grown in a controlled environment and provided 1, 3, or 6 mM nitrate, nitrogen limitation at 1 mM caused a simultaneous rise in foliar levels of phosphate, sucrose, and starch (Robinson, 1997). Under controlled conditions, nitrate increased from dusk to dawn, whereas malate and soluble sugars increased (Steingrover et al., 1986c). There was no change in total osmotic potential in response to changes in sugar concentration in spinach, as there is in lettuce.

We did various experiments to examine the role of irradiance and temperature on the composition of lettuce (Gent, 2014, 2015). Here, we describe the effect of similar studies of composition in spinach grown in hydroponics in a greenhouse. We grew five plantings at different times of year and harvested them in the morning and afternoon to study the effect of irradiance on spinach in hydroponics. We also harvested one planting in summer at 3-h intervals to determine the effects of time of day on metabolites in spinach. We show that spinach metabolism differs from that in lettuce, but it does not affect nitrate concentration.

Materials and Methods

Growth conditions. Plants were grown in Hamden, CT (lat. 42° N long. 73° W, 50 m above sea level) in hoop-houses, 17.1 × 4.3 × 2.7 m high, covered with an inflated, double-glazed 0.1-mm film of clear polyethylene. Heating and ventilating set points were 17 and 22 °C, respectively. From mid-June to September, the houses were covered with 30% shade cloth and the sides were left open. Each of the two greenhouses had two hydroponic systems to supply growing troughs with a 1.5% slope from inlet to outlet. Each of the two systems had 12 troughs running north to south. These were placed alternately along the east-to-west length of the house. Each system had a reservoir into which water and nutrient concentrates were injected automatically to maintain a constant system volume and EC. The dilute solution had initial concentrations of 8 mM nitrate, 4 mM potassium, and adequate concentrations of all other essential elements. A more detailed description is given in previous reports (Gent, 2012a, 2014). Shaded thermocouples and temperature humidity transmitters (model 500; Campbell Scientific, Logan, UT) measured air temperature at a height of 1.5 m in the center of each greenhouse. Quantum sensors (model 190; LI-COR, Lincoln, NE) measured photosynthetic active radiation (PAR) within the greenhouse above the height of the crop. Another sensor measured ambient sunlight in an unobstructed location outside. Readings from each sensor were taken every minute, averaged hourly, and

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recorded. DLI and mean temperature were determined for 5 d before each harvest.

Plant material. Spinach seeds cv. Tye were germinated in an artificial medium (Oasis cubes, model LC1 Horticultures; Smithers Oasis, Kent, OH) under controlled conditions. Seedlings were transplanted into the hydroponic systems 2 weeks after germination. Five plantings were harvested in the interval from June to Nov. 2009 to determine the effect of irradiance. Plants were harvested 3–4 weeks after transplanting in summer and 5.5 and 8 weeks in the fall. Harvests were at 1400 to 1500 HR and at 0700 to 0800 HR the following day. A final planting was harvested 3.5 weeks after transplanting on 25 June 2010. These plants were harvested at 3-h intervals to study the diurnal variation of metabolites. The first three of the harvests in 2009 were from a single greenhouse. The other two harvests in 2009 and the diurnal harvest in 2010 were from two greenhouses. Each planting occupied four troughs per system. Each trough had 14 plants at a density of 25 plants per m² before the harvest commenced. One replicate sample at each harvest was four or eight plants selected from

different troughs and positions along the trough. The selected plants had neighbor plants on all sides. Whole plants were weighed. The Oasis cubes used for germination were cut off, as were the roots below the cube. The roots were washed in tap water, dried by centrifugal force in air, weighed, and placed on dry ice. The shoot was then divided in halves or quarters, depending on plant size. One half or quarter was used for tissue analysis. One portion was weighed and put on dry ice, and later lyophilized, and separated into petioles and leaf blades. These tissues were reweighed, and ground to pass a 20-mesh sieve. The other portion was used to determine leaf area and water content. This sample was separated into individual leaves. Each petiole was excised with a razor blade. The leaf blade was weighed and run through an area meter (Model 3000; LI-COR) to determine the leaf area. The petiole was weighed. Leaf blade and petiole samples were dried at 70 °C and reweighed.

Analyses. Total nitrogen and elemental composition were determined from dried samples hydrolyzed in sulfuric acid and hydrogen peroxide as described previously

(Gent, 2012a). Nitrate, phosphate, malic and oxalic acids, amino acids (except proline), and soluble sugars were extracted and analyzed using liquid chromatography as described previously (Gent, 2012b). The five plantings in 2009 were combined for analysis of metabolite concentrations, with the two or four hydroponic systems used as replicate plots within each planting to determine the effects of irradiance, temperature, and time of day. NDLI was DLI for 5 d before harvest divided by leaf area index. The DLI and NDLI were equal if the index was less than one (Gent, 2014). Fresh and dry mass and leaf area were analyzed by regression vs. NDLI and temperature. Concentrations of metabolites were reported on a fresh mass basis. Dry mass ratio and metabolites were subjected to regression using time of day, morning or afternoon, and NDLI or temperature, and the interaction between these variables. Plants harvested from each of four replicate systems in June 2010 at 3-h intervals were analyzed by regression vs. time of day to determine the variation in metabolites within 1 d. Regressions were done in SYSTAT (SPSS Version 10, Richmond, CA), separately for the shoot

Table 1. Effects on fresh and dry weight and leaf area of spinach of NDLI and temperature.

DLI ^z (mol·m ⁻² ·d ⁻¹)	NDLI ^y (mol·m ⁻² ·d ⁻¹)	Temperature (°C)	Fresh wt (g/plant)	Dry wt (g/plant)	Leaf area index (m ² ·m ⁻²)	Leaf area ratio (m ² ·g ⁻¹)	Specific leaf area (m ² ·g ⁻¹)
4.3	3.0	16.1	66.5	8.38	1.80	86	147
13.2	9.3	17.1	62.0	7.74	1.63	84	142
13.6	13.6	20.1	23.3	3.41	0.86	100	204
29.3	20.5	22.0	47.4	5.35	1.87	140	232
26.6	26.6	23.5	28.8	3.21	1.01	124	197
Significance							
NDLI (N)			0.014	0.006	0.010	NS	NS
Temperature (T)			0.001	0.001	0.001	NS	0.033
N × T			0.001	0.001	0.001	NS	NS

^zDaily light integral.

^yNormalized daily light integral is daily light integral divided by leaf area index, if leaf area index is greater than 1.

Table 2. Effects on dry/fresh mass ratio and composition of spinach shoots on a fresh mass basis of time of day, NDLI, and temperature.

NDLI ^z (mol·m ⁻² ·d ⁻¹)	Temperature (°C)	Dry/fresh mass ratio (g·kg ⁻¹)	Total nitrogen	Nitrate	Amino acids	Malic	Oxalic	Starch	Sugars	K	P	Ca
			mmol·kg ⁻¹									
Morning ^y												
3.0	16.1	113	401	27.4	6.9	2.8	15.1	10	17.8	266	38	64
9.3	17.1	107	392	32.7	5.5	2.9	21.2	33	17.3	244	36	57
13.6	20.1	105	378	13.9	5.5	4.9	12.7	34	5.0	286	27	55
20.5	22.0	103	336	11.2	6.7	8.9	20.7	62	9.6	228	22	36
26.6	23.5	103	318	15.6	5.6	2.9	22.1	71	8.3	275	28	42
Afternoon												
3.0	16.1	115	401	27.1	6.1	2.7	17.0	29	20.7	262	35	65
9.3	17.1	118	390	24.1	6.9	2.8	23.4	63	24.0	273	35	55
13.6	20.1	118	388	11.2	12.6	2.5	19.0	114	9.7	266	25	58
20.5	22.0	126	378	9.8	8.6	4.5	20.7	135	15.7	269	30	43
26.6	23.5	131	396	15.9	9.1	3.3	20.4	132	11.7	328	31	51
Significance												
Time of day (D)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NDLI (N)		NS	0.004	0.002	NS	NS	NS	0.001	0.008	NS	0.007	0.001
D × N		0.001	0.013	NS	NS	NS	NS	0.020	NS	NS	NS	NS
Multiple R ^{2x}		0.84	0.61	0.46	0.39	0.10	0.19	0.91	0.45	0.35	0.44	0.65
Time of day (D)		0.005	0.034	NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature (T)		NS	0.004	0.001	NS	NS	NS	0.001	0.001	NS	0.001	0.001
D × T		0.001	0.016	NS	NS	NS	NS	0.006	NS	NS	NS	NS
Multiple R ²		0.82	0.60	0.59	0.43	0.12	0.12	0.92	0.55	0.31	0.53	0.62

^zNDLI, normalized daily light integral is daily light integral divided by leaf area index, if LAI is greater than 1.

^yMorning harvest from 0700 to 0800 HR, and afternoon harvest from 1400 to 1500 HR.

^xMultiple R² for regression vs. time of day, NDLI or temperature, and their interaction.

and its components, leaf blade, petiole, and roots.

Results

Effect of normalized DLI on dry matter and metabolites. The DLI was 13.6 and 26.6 mol·m⁻²·d⁻¹ for the two harvests in June 2009, 29.3 mol·m⁻²·d⁻¹ in July, 13.2 mol·m⁻²·d⁻¹ in October, and 4.3 mol·m⁻²·d⁻¹ in November (Table 1). The DLI in October and November was much lower than that in June and July. Leaf area index varied from 0.9 to 1.9 m²·m⁻². In summer, the leaf area to shoot mass ratio, 100 to 140 cm²·g⁻¹, and the specific area to mass ratio of leaf blades, 197 to 232 cm²·g⁻¹, were greater than in the fall, 84 to 86 cm²·g⁻¹ and 142 to 147 cm²·g⁻¹, respectively. The NDLI ranged from 3.0 to 26.6 mol·m⁻²·d⁻¹. For the diurnal harvest in 2010, DLI was 23.6 mol·m⁻²·d⁻¹. The NDLI was 16.1 mol·m⁻²·d⁻¹ and temperature was 23 °C. For the harvests in 2009, NDLI and temperature were highly correlated with various metabolites and with each other, $R^2 = 0.97$, so regression could not use both variables. Analysis of variance was done separately using NDLI or mean temperature. We noted where the significance of effects of temperature was greater than those of NDLI.

The regression of dry/fresh mass ratio of spinach shoots, expressed as grams dry mass/kilogram fresh mass, had an interaction between the effects of NDLI and time of day (Table 2). The dry/fresh mass ratio changed with NDLI and the interaction between NDLI and time of day in leaf blades and petioles, but not in roots (Fig. 1). Root dry/fresh mass ratio averaged 60 g·kg⁻¹ and petioles had ≈110 g·kg⁻¹. Dry/fresh mass ratio in blades was 110 g·kg⁻¹ in the morning, but increased in the afternoon to 140 g·kg⁻¹ under high NDLI. That in petioles remained constant in the afternoon, but declined in the morning under high NDLI. There were no changes under low irradiance.

Reduced N in spinach shoots was higher in the afternoon than in the morning under NDLI greater than 20 mol·m⁻²·d⁻¹ (Table 2). Shoot nitrogen was 390 mmol·kg⁻¹ in the afternoon, but it was as low as 318 mmol·kg⁻¹ in the morning under high irradiance. Regressions were significant for NDLI in petioles, and for the interaction between NDLI and time of day in petioles and blades (data not shown). Reduced N was 200 mmol·kg⁻¹ in roots. Petioles varied from 280 mmol·kg⁻¹ under low NDLI to 170 mmol·kg⁻¹ under high NDLI in the morning. Reduced N in blades was 440 mmol·kg⁻¹ under low NDLI in morning or afternoon, and 380 and 480 mmol·kg⁻¹ in at high NDLI in morning or afternoon, respectively.

Nitrate in spinach shoots did not change from morning to afternoon, but decreased from 27 to 10 mmol·kg⁻¹ as NDLI increased to 13 mol·m⁻²·d⁻¹ or temperature increased to 20 °C (Table 2). Temperature and NDLI were highly correlated, but temperature had a greater R^2 for regression than NDLI, multiple $R^2 = 0.59$ for temperature and $R^2 = 0.46$ for NDLI. Examining individual parts of the

Fig. 1. Dry/fresh mass ratio of spinach as a function of normalized daily light integral (NDLI). Symbols represent leaf blade, petiole, and root, and time of harvest at AM or PM. Bars indicate the least significant difference at $P < 0.05$. Lines indicate linear trends with irradiance for morning or afternoon data.

Fig. 2. Nitrate concentration in spinach on a fresh weight basis as a function of normalized daily light integral (NDLI). Symbols represent leaf blade, petiole, and root, and time of harvest at AM or PM. Bars indicate the least significant difference at $P < 0.05$. Lines indicate quadratic trends with irradiance for morning or afternoon data.

plant showed nitrate changed in both petioles and blades (Fig. 2). The nitrate in roots was 12–25 mmol·kg⁻¹. Nitrate in petioles was 25 to 40 mmol·kg⁻¹ in the morning, but fell to 20 mmol·kg⁻¹ in the afternoon when temperature was greater than 20 °C. Nitrate in blades was 25 to 30 mmol·kg⁻¹ under low temperature, but decreased to 6 to 8 mmol·kg⁻¹ when temperature was greater than 20 °C. Under each temperature, nitrate

in petioles was slightly higher in the morning than afternoon.

Amino acids did not change appreciably with NDLI or time of day in spinach shoots (Table 2). There was an effect in blades, with effects of temperature and the interaction of temperature and time of day more significant than that of NDLI, multiple $R^2 = 0.53$ for temperature and $R^2 = 0.46$ for NDLI (data not shown). Amino acids were 2–4 mmol·kg⁻¹ in

roots and 5–7 mmol·kg⁻¹ in petioles. Amino acids did not change in blades in the morning, but increased in the afternoon from 4 to 8 mmol·kg⁻¹ at high temperature. The amount of glutamine in petioles decreased with normalized irradiance. Very little glutamine was found in blades or roots.

Regression vs. temperature or NDLI and the interaction with time of day had effects on starch in shoots (Table 2). Starch in petioles and blades increased in morning or afternoon to the highest irradiance measured, 27 mol·m⁻²·d⁻¹ (Fig. 3). Only NDLI or temperature had an effect on starch in roots. Starch in roots was between 5 and 15 mmol glucose kg⁻¹. Starch in petioles increased with irradiance from 20 to 80 mmol·kg⁻¹ in the morning to 30 to 120 mmol·kg⁻¹ in the afternoon. Starch in blades increased with irradiance from 5 to 60 mmol·kg⁻¹ in the morning to 30 to 140 mmol·kg⁻¹ in the afternoon.

However, the growth environment had an opposite effect on free sugars. The effect of temperature was greater than that of NDLI, multiple $R^2 = 0.55$ for temperature and $R^2 = 0.45$ for NDLI. Sugars in the shoot did not change between morning and afternoon (Table 2). Sugars in roots and blades were 4–6 mmol·kg⁻¹ and 10–15 mmol·kg⁻¹, respectively (Fig. 4). There was a greater effect of temperature than NDLI on sugars in petioles, multiple $R^2 = 0.80$ for temperature and $R^2 = 0.70$ for NDLI. Sugars in petioles decreased from 50 to 60 mmol·kg⁻¹ at low temperature to less than 20 mmol·kg⁻¹ at temperature greater than 20 °C.

There was no relationship between irradiance and the concentrations of malate in shoots of spinach or in the individual parts. Malate was ≈5 mmol·kg⁻¹ in roots, 8–12 mmol·kg⁻¹ in petioles, and 20–25 mmol·kg⁻¹ in blades. Although there was no relationship between irradiance and the concentrations of oxalate in shoots, oxalate in blades showed an increase due to irradiance and temperature, but not due to time of day. Oxalate was 6–10 mmol·kg⁻¹ in roots and 12–18 mmol·kg⁻¹ in petioles. Oxalate in blades increased from 8 to 14 mmol·kg⁻¹ under high NDLI or high temperature.

Potassium in spinach had no dependence on irradiance or temperature and time of day (Table 2). Values were 20–25 mmol·kg⁻¹ in roots and 250–300 mmol·kg⁻¹ in petioles and blades. Both P and Ca depended on NDLI or temperature but not time of day. For P, the effect of temperature was more significant than that of NDLI; multiple was $R^2 = 0.53$ for temperature and $R^2 = 0.44$ for NDLI. Phosphorus did not vary in shoots between morning and afternoon, but values were lower under high temperature. Values were 27 and 22 mmol·kg⁻¹ in roots, 35 and 28 mmol·kg⁻¹ in petioles, and 37 and 28 mmol·kg⁻¹ in blades at low and high temperature, respectively. The same effect was seen for Ca, but effects of temperature and NDLI were similar. The Ca concentrations were 10 mmol·kg⁻¹ in roots, 20

Fig. 3. Starch concentration as glucose in spinach on a fresh weight basis as a function of normalized daily light integral (NDLI). Symbols represent leaf blade, petiole, and root, and time of harvest at AM or PM. Bars indicate the least significant difference at $P < 0.05$. Lines indicate quadratic trends with irradiance for morning or afternoon data of leaf and petiole, and linear trends for root.

Fig. 4. Soluble sugar concentration as glucose in spinach on a fresh weight basis as a function of normalized daily light integral (NDLI). Symbols represent leaf blade, petiole, and root, and time of harvest at AM or PM. Bars indicate the least significant difference at $P < 0.05$. Lines indicate quadratic trends for morning or afternoon data for petiole, and linear trends for leaf and root.

to 40 mmol·kg⁻¹ in petioles, and 50 to 70 mmol·kg⁻¹ in blades at low and high NDLI or temperature, respectively.

Diurnal variation of metabolites in spinach. When harvested at 3-h intervals in 2010, the dry/fresh mass ratio of spinach shoots varied with time of day from 91 to 114 g·kg⁻¹ (Table 3). First-, second-, and third-order terms in time of day were

significant. The dry/fresh mass ratio differed among parts of spinach. Roots had 55 g·kg⁻¹ at 0800 HR and 60 g·kg⁻¹ at 1800 HR (Fig. 5). Petioles had 80 g·kg⁻¹ at 0800 HR increasing to 90 g·kg⁻¹ at 1800 HR. The blade dry/fresh mass ratio was 95 g·kg⁻¹ at 0800 HR, increasing to 120 g·kg⁻¹ at 1500 HR. The values in petioles and blades decreased from 0500 to 0800 HR and from 1800 to 2100 HR.

Table 3. Effects on dry/fresh mass ratio and composition in spinach shoots on a fresh mass basis of linear, quadratic, and cubic terms in time of day.

Time (HR)	Dry/fresh mass ratio (g·kg ⁻¹)	Nitrate	Malic	Oxalic	Starch	Sugars	Sucrose	Glucose	Fructose	P
		mmol·kg ⁻¹								
0500	97	23	7.3	29.8	34.5	3.7	1.5	1.4	0.7	7.7
0800	91	24	7.3	21.4	22.4	3.2	1.6	0.9	0.8	6.1
1100	98	37	8.5	33.0	32.9	6.6	2.9	2.1	1.6	12.5
1300	110	27	7.6	35.3	52.2	10.8	4.0	3.8	3.1	12.3
1500	114	27	6.2	34.4	66.9	10.5	3.8	3.6	3.1	14.3
1800	114	24	9.0	32.0	81.8	10.0	5.6	3.0	1.4	7.3
2100	108	33	7.2	33.7	69.9	5.8	2.8	2.1	1.0	15.2
		Significance ^z								
Time	L***	NS	NS	NS		L**	L**	L*	NS	NS
	Q***				L***, Q***	Q***	Q**	Q*		
	C***				C***	C***	C**	C**		

^zThe various components of spinach shoots were regressed against linear, quadratic, and cubic orders in time.

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

Nitrate content of spinach shoots varied from 23 to 37 mmol·kg⁻¹, but this variation had no relation to time of day. The nitrate concentration varied from 37–56 mmol·kg⁻¹ in petioles and 18–30 mmol·kg⁻¹ in blades, with no effect of time of day. The nitrate in roots decreased during the day from 50 to 30 mmol·kg⁻¹ (Fig. 6).

Starch concentration in spinach shoots showed a diurnal variation with a minimum at 0800 HR and a maximum at 1800 HR (Table 3). The starch in roots changed only slightly during the day from 10 to 15 mmol·kg⁻¹ (Fig. 7). Starch in petioles increased from 20 to 35 mmol·kg⁻¹ during the day, while in blades it increased from 20 to 100 mmol·kg⁻¹. The most rapid change in starch was at the time of highest irradiance from 1000 to 1600 HR. Starch in blades also decreased from 0500 to 0800 HR and from 1800 to 2100 HR.

There was a diurnal variation of sugars in shoots of spinach (Table 3). The individual components, sucrose, glucose, and fructose, had a binary response to irradiance or temperature, with high values in the day and low values in the night. The sum of sugars in roots were 4 mmol·kg⁻¹ at night and 6 mmol·kg⁻¹ in the day (Fig. 8). In petioles, the values were 6 mmol·kg⁻¹ at night and 15 to 18 mmol·kg⁻¹ in the day. The highest values were from 1300 to 1800 HR under high irradiance. Values in blades were 2 mmol·kg⁻¹ at night and 6 to 8 mmol·kg⁻¹ in the day. The values in roots and blades also decreased from 0500 to 0800 HR and from 1800 to 2100 HR.

Second-order effects of time were significant for oxalate in blade, petiole, and roots (Fig. 9). The blades and petioles had higher values at 2100 HR compared with 1700 HR. We did not follow oxalate through the night, but it was low at dawn, and decreased until midday. The oxalate content averaged 6.3, 9.5, and 5.6 mmol·kg⁻¹ in blades, petioles, and roots, respectively. There was no diurnal variation in malic acid (data not shown). Neither potassium and calcium nor phosphorus had a response to time of day.

Discussion

The NDLI affected dry/fresh mass ratio only in leaf blades and petioles of spinach. Young blades had more dry mass than old blades or petioles (Beis et al., 2002). The

Fig. 5. Dry/fresh mass ratio of spinach as a function of time of day. Symbols represent leaf blade, petiole, and root. Bars indicate the least significant difference at $P < 0.05$. Lines indicate cubic trends with time for each part.

increase in dry/fresh mass ratio at high NDLI may be due to more young leaves on plants in summer. The plants harvested in summer were younger than those in fall. The dry/fresh mass ratio increased with NDLI in all parts of lettuce plants (Gent, 2012a). In the afternoon under high irradiance, dry/fresh mass ratio of small lettuce plants and spinach was equal. Osmotic potential is constant in spinach due to nitrate uptake and starch degradation at night (Steingrover et al., 1986c). In lettuce, carbohydrate from photosynthesis is stored as soluble carbohydrate (Blom-Zandstra and Lampe, 1985), whereas in spinach it is starch. Due to leaf turgor, leaf expansion of spinach was low in the afternoon (Steingrover et al., 1986c).

Spinach had more reduced N in blades, and especially in petioles, than lettuce, except under high irradiance. The difference in reduced N was greater in afternoon than in morning at high compared with low NDLI. Spinach had less N in the morning due to more starch at high compared with low

NDLI. Reduced-N concentrations in spinach blades and petioles were greater than those in lettuce under low light conditions, although values of nitrate in lettuce grown under the same conditions were about the same as in spinach (Gent, 2014). Scaife and Schloemer (1994) reported that nitrate in the shoot increased from 80 to 220 mmol·kg⁻¹ over a 14-h dark period in a controlled environment. In another experiment with spinach under low light, the nitrate concentrations were 42 and 13 mmol·kg⁻¹ in blades and 200 and 195 mmol·kg⁻¹ in petioles at dawn and dusk, respectively (Steingrover et al., 1986a). These authors reported higher values in petioles than we found for a diurnal harvest in summer, but similar values in blades. We found more nitrate in petioles, but less diurnal variation than in blades. Several authors have shown that supplemental light reduces nitrate in spinach at night (Chadja et al., 1999; Fukuda et al., 1999; Ohashi-Kaneko et al., 2007; Steingrover et al., 1986b). High irradiance lowered nitrate in blades in

Fig. 6. Nitrate in spinach as a function of time of day. Symbols represent leaf blade, petiole, and root. Bars indicate the least significant difference at $P < 0.05$. Lines indicate quadratic trends with time for each part.

Fig. 7. Starch as glucose in spinach as a function of time of day. Symbols represent leaf blade, petiole, and root. Bars indicate the least significant difference at $P < 0.05$. Lines indicate cubic trends with time for each part.

morning and afternoon. Spinach does not have to take up nitrate at the maximum rate to maintain yield (Steingrobe and Schenk, 1991).

The amino acids in blades of spinach were 2-fold higher in afternoon compared with morning, under high compared with low NDLI or temperature. Temperature had a greater effect than NDLI. When spinach was switched to no nitrate, free sugars increased more in

blades than roots, but amino acids declined more in roots than blades (Buysse et al., 1996): under low light, sugars and amino acids decreased for 1 to 2 d then they were stable. The doubling of amino acid concentration with NDLI in blades was one specific difference in composition of spinach compared with lettuce. It may be one reason why spinach had a higher amount of reduced nitrogen than that of lettuce.

Starch changed with NDLI or time of day in spinach. Starch increased throughout the day and decreased at night. Blades had the greatest change. There may be a relationship between sugar and starch concentrations. Starch increased with NDLI in both morning and afternoon. However, sugars decreased with temperature due to a decrease in petioles. Sugar concentrations may increase when starch is low. Starch formation or translocation may deplete the concentration of sugars in blades in the afternoon. We found the concentration of sugars in petioles was more sensitive to temperature than to NDLI. In other species, temperature plays a larger role in sugar concentration than does irradiance (Yamori et al., 2010). The diurnal pattern of sugars in lettuce (Gent, 2012a) was very similar to that of starch in spinach.

Sugars are not an osmoticum affecting nitrate content of spinach, where sugar is rapidly converted to starch, although has been proposed as such for lettuce (Blom-Zandstra and Lampe, 1985). The amount of nitrate is similar in both species. There are changes in P and Ca in lettuce but not in spinach (Burns et al., 2011; Gent, 2014). These other soluble minerals may help account for the changes in dry/fresh mass ratio of lettuce. The concentrations of P changed with temperature in spinach, but not with NDLI or time of day. Increasing root-zone temperature from 20 to 28 °C increased leaf weight of spinach in summer (Ikeda et al., 1995). In their study, P increased across the range of temperatures in summer. However, we observed high P only under cool temperature.

Our studies showed no change in oxalate with time of day, but it increased with irradiance and temperature (Table 2). In the diurnal study, the concentration in petioles was 2-fold higher than in blades (Fig. 9), and the lowest oxalate was at 1200 HR and the highest oxalate was at 2100 HR. We did not follow oxalate through the night, but it decreased at 0500 HR. Our results differed from the results published by other authors, who found more nitrate and oxalate under low light (Proietti et al., 2004). Likewise, in a study of 182 cultivars of spinach grown in four seasons in the field, oxalate was lower in summer than in winter (Kaminishi and Kita, 2006). One cultivar showed more oxalate in old blades (Beis et al., 2002). However, others did not observe a correlation between in oxalate in winter and summer (Kawazu et al., 2003). More oxalate was found in blades than in petioles (Zhang et al., 2009). Our studies of oxalate were more like those of beta-carotene concentrations in spinach, which showed a decrease at dawn then increasing and stayed high until night (Oyama et al., 2000). This aspect of spinach composition needs more study.

The change of concentrations of starch, sugars, reduced N, and nitrate due to time of day on a fresh mass basis may be due to differences in content on a dry mass basis. However the concentration of starch in the diurnal experiment changed by 5-fold,

Fig. 8. Sugars as glucose in spinach as a function of time of day. Symbols represent leaf blade, petiole, and root. Bars indicate the least significant difference at $P < 0.05$. Lines indicate cubic trends with time for each part.

Fig. 9. Oxalic acid in spinach as a function of time of day. Symbols represent leaf blade, petiole, and root. Bars indicate the least significant difference at $P < 0.05$. Lines indicate cubic trends with time for each part.

compared with a mere change of 25% in dry/fresh mass ratio of blades over the day. Although the change in dry/fresh mass ratio can explain some of the change in starch, it cannot explain the full effect. There would be greater effects of time of day on nitrate on a dry mass, compared with a fresh mass basis, as nitrate tends to decrease in the afternoon. However even in this case, the plant takes up and transpires water as needed to keep metabolite concentrations in the desired range.

Under low light conditions, there are diurnal changes of nitrate and sugars in spinach shoots (Scaife and Schloemer, 1994; Steingrover et al., 1986a). However, when compared across a range of NDLI from summer to fall, we found the effect of temperature was greater than that of time of day. Under low temperature, shoots had high nitrate and sugars and under high temperature, they had low values. This suggests the growth of spinach may be slow in fall, compared with

summer, due to a lower temperature in the fall, which decreases the metabolism of sugars and nitrate. As in lettuce (Gent, 2003), spinach may benefit from higher concentrations of nitrate in the nutrient solution when grown in summer compared with fall.

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