Light Intensity and Time of Day at Harvest Affects Ascorbic Acid Concentration and Mineral Nutrient Content and Leaf Greenness in Field-grown Mustard Greens

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ABSTRACT

Field-grown mustard greens, *Brassica juncea* (L.), were used to validate several observations of a greenhouse study which reported nutrient changes in mustard greens grown, in part, under ambient and reduced light. Seedlings of the cultivar Florida Broadleaf were transplanted into a Hidalgo sandy clay soil near Weslaco, TX (26º 08' Lat.) on 6 Nov. 03. At 14 days prior to harvest, the following light regimes were established: (1) continuous ambient light; (2) 7 days of 50% shade then 7 days of ambient light; (3) 7 days of ambient light then 7 days of 50% shade; and (4) 14 days of 50% shade. Cumulative solar radiation was 28.9 and 19.4 $kW{\cdot}m^{-2}$ during the first and second 7 days, respectively. Cumulative light during the 14-day period, as photosynthetic photon flux density (PPFD), was 101, 67, 78, and 44 $mMol{\cdot}s^{-1}{\cdot}m^{-2}$ for treatments 1-4 , respectively. Plants were harvested at 0800, 1100, and 1400 hrs on 2 Jan. 04. Shade during the last 7 days prior to harvest generally had the greatest effect on leaf chemical composition. Increased shade duration did not significantly affect the agronomic performance, but did increase leaf total carotenoids, chlorophylls, water content, most mineral nutrients (dry wt. basis), and reduced total ascorbate levels (fresh wt. basis). As daylight progressed, sample plant wt. and avg. leaf wt. decreased in shaded plants only. Free ascorbic acid, NO_3, Mg, chlorophyll a:b ratio, and the chlorophyll to carotenoid ratio decreased with time of day. Cumulative sunlight, as PPFD, was significantly correlated with total ascorbate, chlorophyll a:b ratio, and plant wt. ($P < 0.06$) and negatively correlated with NO_3, Fe, chlorophylls, and total carotenoids. Thus, cloudy weather prior to harvest and, to a lesser extent, the time of day can affect leaf Vitamin C, mineral nutrients, and alter leaf greenness.

RESUMEN

Se realizó un estudio de campo con plantas de mostaza, *Brassica juncea* (L.), para validar observaciones previas de cambios en la concentración de nutrientes en plantas de mostaza creciendo en invernadero bajo condiciones de luz ambiental y luz reducida. Se transplantaron plántulas del cultivar Florida Broadleaf en suelo arcilloso-arenoso cerca de Weslaco, Texas (Lat. 26º 08') el 6 de noviembre del 2003. Catorce días antes de la cosecha, se establecieron los siguientes regímenes de luz: (1) luz ambiental continua; (2) 7 días de 50% sombra seguido de 7 días de luz ambiental; (3) 7 días de luz ambiental seguido de 7 días de 50% de sombra; y (4) 14 días de 50% de sombra. La radiación solar acumulada fue 28.9 y 19.4$kW.m^{-2}$ durante los primeros y segundos periodos de 7 días respectivamente. La luz acumulada durante el periodo de 14 días, medido como densidad de flujo de fotón fotosintético (PPFD), fue 101, 67, 78, y 44 $mMol{\cdot}s^{-1}{\cdot}m^{-2}$ para los tratamientos 1-4 respectivamente. Las plantas se cosecharon a las 08:00, 11:00, y 14:00 horas del 2 de enero del 2004. El sombreado durante los últimos 7 días previos a la cosecha generalmente tuvo el mayor efecto en la composición química de la hoja. El incremento de la duración del sombreado no afectó significativamente el comportamiento agronómico, pero incrementó el contenido total de carotenoides, clorofilas, agua, nutrientes minerales (en base al peso seco) y redujo los niveles totales de ascorbato (en base a peso fresco) en la hoja. A medida que progresó la luz del día, el peso de la planta muestreada y el peso promedio de la hoja disminuyeron solamente en las plantas sombreadas. El ácido ascórbico libre, NO_3, Mg, la relación clorofila a:b, y la relación clorofila:carotenoide disminuyó con la hora del día. La luz del sol acumulada, expresada como PPFD, se correlacionó significativamente con el ascorbato total, la relación clorofila a:b, y el peso de la planta ($P < 0.06$) y se correlacionó negativamente con el NO_3, Fe, clorofilas y carotenoides totales. De acuerdo a lo observado, el clima nublado previo a la cosecha y, en menor medida, la hora del día, pueden afectar la vitamina C y nutrientes minerales de la hoja y alterar el color verde de ésta.

Key Words: Vitamin C, shading

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Leafy greens are an excellent source of mineral nutrients and vitamins for the human diet. Growing environment and cultural practices can affect the accumulation of nutrients in (leafy) vegetables (Mozafar, 1994). Previous research has demonstrated that vegetable amaranth grown under reduced light accumulates higher levels of most mineral nutrients, NO_3, leaf protein, carotenoids, and soluble oxalates (Makus and Hettiarachchy, 2001). Decreasing light levels increases area per leaf, mineral nutrients, and pigments (dry wt. basis) of snap bean leaves (Makus, 2001). 'Carlo' snap beans, when grown under reduced light, had higher dry matter (%) and Mn levels but lower Fe levels in sieve size No. 3 pods (Makus, 2001). High light intensity prior to asparagus harvest, increased spear ascorbate content, whereas cool pre-harvest temperatures increased spear soluble solids concentration (Makus, 1992). In a recent greenhouse study, we have observed that greens grown under reduced light produce higher levels of plant pigments (dry wt. basis), accumulate more mineral nutrients (dry wt.

Table 1. Effect of pre-harvest light regime and time of harvest on agronomic responses, leaf water content, ascorbic acid, and pigments of mustard greens harvested 2 January 2004. Treatments prior to harvest were (1) continuous ambient light; (2) 7days of 50% shade then 7 days of ambient light; (3) 7 days of ambient light then 7 days of 50% shade; and (4) 14 days of 50% shade.

				Sample	Ascorbate		Pigments			
	Plant wt	Leaves/ Plant	Leaf wt	Water Content	Free	Total	Total Chloro	a:b ratio	Carot	Chloro:Carot ratio
	(kg)		(g)	(%)	mg/100g fr wt		mg/g dry wt			
Treatment:										
1	1.354 a	14.2 a	95.3 a	90.4 b	32.1 a	96.8 a	10.67 b	2.60 a	2.51 b	4.25 bc
2	1.212 a	12.2 a	100.2 a	90.4 b	25.9 b	93.2 ab	10.71 b	2.58 a	2.54 b	4.22 c
3	1.314 a	13.4 a	98.6 a	92.1 a	22.0 bc	77.0 bc	11.77 ab	2.40 b	2.72 ab	4.33ab
4	1.104 a	12.7 a	89.2 a	92.8 a	19.8 c	67.3 c	12.94 a	2.27 c	2.97 a	4.36 a
	NS[Z]	NS	NS	**	**	**	**	**	**	**
Contrast:										
1&2 vs. 3&4	NS	NS	NS	**	**	**	**	**	**	**
Time of day										
0800	1.431 a	13.4 a	108.2 a	91.9 a	23.4 b	83.1 a	11.61 a	2.57 a	2.77 a	4.18 c
1100	1.207 b	12.8 a	96.0 ab	91.6 a	23.7 b	85.9 a	11.17 a	2.44 b	2.61 a	4.28 b
1400	1.100 b	13.2 a	83.3 b	90.8 a	27.8 a	81.7 a	11.79 a	2.38 b	2.67 a	4.42 a
	**	NS	*	NS	*	NS	NS	**	NS	**
Interactions:										
TRT X Time	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

[Z] NS, *, ** = not significant and significant at $P<0.05$ and $P<0.01$, respectively. Main effect column means followed by the same letter are not significantly different at the probability shown.

Table 2. Effect of light intensity and time of harvest on leaf N, total cations and macro-nutrients from mustard greens harvested 2 January 2004.

Main effects[Z]	N	Total cations	K	S	P	Mg
	% dry wt					
Treatment:[Y]						
1	5.17 b	8.04 b	4.51 b	0.861 b	0.290 b	0.170 a
2	5.10 b	8.38 b	4.74 b	0.877 b	0.288 b	0.181 a
3	5.78 a	9.43 a	5.54 a	1.010 a	0.362 a	0.170 a
4	5.75 a	9.74 a	5.94 a	1.012 a	0.368 a	0.173 a
	**[X]	**	**	**	*	NS
Contrast:						
1&2 vs 3&4	**	**	**	**	**	NS
Time of day						
0800	5.50 a	8.96 a	5.21 a	0.924 a	0.332 a	0.180 a
1100	5.49 a	9.10 a	5.40 a	0.982 a	0.346 a	0.177 ab
1400	5.35 a	8.62 a	4.93 a	0.914 a	0.303 a	0.164 b
	NS	NS	NS	NS	NS	*

[Z]There were no treatment x time interactions. Ca concentration (1.84 %) was not affected by treatment or time.

[Y]Treatment code same as in Table 1 legend.

[X]NS, *, ** = not significant and significant at $P<0.05$ and $P<0.01$, respectively. Main effect column means followed by the same letter are not significantly different at the probability shown.

Table 3. Effect of light intensity and time of harvest on leaf NO_3 and mineral micro-nutrients from mustard greens harvested 2 January 2004.

Main effects[Z]	NO_3	Na	Al	Fe	Mn	B	Cu
	μg/kg dry wt						
Treatment:[Y]							
1	4218 b	3921 a	130 b	164 c	50.8 b	30.8 c	12.3 a
2	4968 b	4216 a	169 ab	200 bc	58.1 ab	32.2 bc	12.5 a
3	7676 a	4164 a	226 a	239 ab	52.9 b	34.7 ab	13.3 a
4	8344 a	3016 b	224 a	247 a	65.0 a	37.8 a	13.5 a
	**[X]	**	**	**	**	**	NS
Contrast:							
1&2 vs 3&4	**	NS	**	**	NS	**	**
Time of day:							
0800	6927 a	4142 a	193 a	227 a	54.4 a	33.3 a	12.8 a
1100	6496 ab	3433 a	192 a	215 a	55.7 a	33.2 a	13.0 a
1400	5482 b	3913 a	176 a	195 a	60.0 a	35.2 a	12.8 a
	*	NS	NS	NS	NS	NS	NS

[Z]There were no treatment x time interactions. Zn concentrations (29.6 μg/kg) were not effected by treatment or time.

[Y]Treatment code same as in Table 1 legend.

[X]NS, *, ** = not significant and significant at $P<0.05$ and $P<0.01$, respectively. Main effect column means followed by the same letter are not significantly different at the probability shown.

basis), have larger leaf areas, but are lower in ascorbic acid concentrations (fresh wt. basis) (Makus and Lester; 2002).

Spring and fall-grown greens are often exposed to different light environments. Light regimes can vary year to year and location to location. The objective of the present study was to evaluate the effects of different simulated cloudy weather conditions two weeks prior to harvest and the effect of time of day on leaf ascorbate, pigments, and mineral nutrients of field-grown 'Florida Broadleaf' mustard greens.

MATERIAL AND METHODS

Seedlings of 'Florida Broadleaf' mustard greens were transplanted into a Hidalgo sandy clay loam (Fine-loamy, mixed, hyperthermic Typic Calciustolls) soil on 6 Nov. 2003 at 0.5 m in-row intervals. Rainfall (38 mm) was supplemented with 64 mm of water by trickle irrigation to plant beds when soil moisture was depleted to -20 kPa at 15 cm. On 12 Dec., 30 kg $N{\bullet}ha^{-1}$ (11-37-0) was added by surface trickle irrigation. Beginning 19 Dec., plants were grown under the following four light regimes: (1) continuous ambient light; (2) 7 days of 50% shade followed by 7 days of ambient light; (3) 7 days of ambient light followed by 7 days of 50% shade; and (4) 14 days of 50% shade. The polypropylene fabric was supported by 50-cm high (mid-point) wire hoops and the edges were heeled into soil at both sides. Temperature and photosynthetic photon flux density (PPFD) were measured with shielded thermocouples and LI-190SA quantum sensors, respectively, at approximately 30 cm height. Cumulative PPFD over the 14-day treatment period was obtained by adding the average hourly PPFD values. Solar radiation, as $kW{\bullet}m^{-2}$, was measured hourly with a LI-200SA pyranometer sensor at the site weather station.

Plants were harvested on 2 January 2004 and weighed. The youngest, fully expanded leaf from each plant was removed for chemical analysis. Selected leaves were then split along the lamina, with one-half used for ascorbate and the opposite half used for pigment and nutrient tests. Three randomly selected plants within each light treatment were harvested at each harvest interval and plant fresh weight, average leaf weight, and number of leaves per plant determined. Approximately 20 g of fresh leaf material were used for ascorbic acid analyses based on the procedure of Lester and Crosby (2002). Approximately 85 g of leaves were frozen, lyophilized, and passed through a 40 mesh (0.36 mm^2) screen with a Wiley Mill. From the latter sub-samples, pigments were analyzed spectrophotometrically by the procedure of Wellburn and Lichtenthaler (1984). Leaf mineral nutrients (K, P, Ca, Mg, S, Na, Al, Fe, Mn, Zn, B, and Cu) were determined, after HNO_3 digest, by ICP spectroscopy (Plank, 1992). Leaf total-N was determined by dry combustion using a LECO FP428 analyzer and NO_3-N, after water extraction, by a Skalar autoanalyzer (Plank, 1992).

The experiment was analyzed as a completely random (N = 3) design with two factors, treatment and time. Treatments consisted of four light regimes and time was based on three within day harvest periods. Differences between response means were tested using the PDIFF option of the LSMEANS statement of PROC GLM of SAS Version 8.2 (SAS Institute, Cary, N.C.).

RESULTS

Environment. During the first and second weeks of the light regime treatments, cumulative solar radiation was 28.9 and 19.4 $kW{\bullet}m^{-2}$, respectively. The cumulative average hourly PPFD for treatments 1 to 4 were 108, 67, 78, and 44 $mMol{\bullet}s^{-1}{\bullet}m^{-2}$, respectively. Mean weekly canopy temperatures in full sun during the first and second weeks, were 16.5 and 18.0ºC, respectively. Average weekly canopy temperature differences between shaded and unshaded plants were <0.2ºC during this

Table 4. Linear correlations between several leaf attributes and cumulative hourly photosynthetic photon flux density (PPFD) during the treatment period; n = 36.

	PPFD	Chloro	Carot	Free ascorbate	Total ascorbate	Total-N	NO_3-N	Fe
PPFD	–	-0.558**	-0.537**	0.616**	0.521**	-0.382*	-0.598**	-0.492**
Chloro		–	0.965**	-0.362*	-0.567**	0.591**	0.565**	0.626**
Carot			–	-0.376*	-0.508	0.594**	0.575**	0.647**
Free ascorbate				–	0.514**	-0.467**	-0.671**	-0.543**
Total ascorbate					–	-0.316	-0.666**	-0.633**
Total-N						–	–0.664**	0.323*
NO_3-N							–	0.701**
Fe								–

interval. On 2 Jan. 2004, the day of harvest, the avg. hourly temperature and PPFD one hour prior to harvesting greens at 0800 hrs for full sun / shaded plants were 20.3 / 20.3ºC and 0.019 / 0.002 $mMol{\cdot}s^{-1}{\cdot}m^{-2}$, respectively. Corresponding conditions for the 1100 hr harvest were 25.5 / 24.9ºC and 0.421 / 0.284 $mMol{\cdot}s^{-1}{\cdot}m^{-2}$, respectively. Corresponding conditions for the 1400 hr harvest were 29.5 / 28.1ºC and 1.023 / 0.474 $mMol{\cdot}s^{-1}{\cdot}m^{-2}$, respectively.

Agronomic. Fresh weight, number of leaves, and average leaf weight per plant were not affected by light treatments (Table 1). Leaf water content was approximately 2% (absolute) higher in leaves exposed to reduced light both one and two weeks prior to harvest. As the harvest day progressed, plant weight and average leaf weight declined, but leaf water content was not significantly affected. Plants exposed to 7 days of shade prior to harvest suffered the largest plant weight loss between 0800 and 1400 hrs compared to the other treatments, thus accounting for the treatment X time interaction (data not shown).

Ascorbic acid and Leaf pigments. Temporary shading one week prior to harvest (treatments 3 and 4) decreased leaf free and total ascorbic acid levels, and the chlorophyll a:b ratio, but increased total chlorophyll, total carotenoids, and the chlorophyll to carotenoid ratio (Table 1). Free ascorbic acid was highest at the early afternoon (1400 hrs) harvest compared to the two morning harvests (0800 and 1100 hrs). The chlorophyll a:b ratio declined, whereas chlorophyll to carotenoid ratio increased over the harvest day (between 0800 and 1400 hrs).

Mineral nutrients. Leaves of greens grown under reduced light for one to two consecutive weeks were higher in total-N, total cations, K, S, and P compared to leaves exposed to ambient light levels prior to harvest (Table 2). Leaf Ca levels were not affected by light regime or time of harvest. Leaf Mg levels were not affected by light regime, but were reduced as the day progressed.

In the case of leaf micro-nutrients, greens grown under reduced light for one to two consecutive weeks prior to harvest were higher in leaf Al, Fe, B, and Cu compared to greens not exposed to reduced light during this same period (Table 3). NO_3 levels were almost twice as high in plants which were shaded one or two consecutive weeks prior to harvest compared to plants which were unshaded prior to harvest. As the day progressed, leaf NO_3 levels declined. However, rate of nitrate loss in plants not receiving shade during the two week period was greater than in treatments receiving shade, thus accounting for the interaction (data not shown).

Correlations. Light level, as cumulative PPFD, was positively correlated with ascorbic acid particularly free ascorbate, but was negatively correlated with total leaf chlorophyll, carotenoids, total-N, NO_3-N, and Fe (Table 4). Leaf ascorbate levels were negatively correlated with leaf chlorophyll, carotenoids, total-N, NO_3-N, and Fe.

DISCUSSION

The effect of reduced light on increased leaf pigments and mineral accumulation has been observed in field-grown vegetable amaranth (Makus and Hettiarachchy, 2001), snap bean leaves (Makus, 2001), and greenhouse-grown mustard greens (Makus and Lester, 2002). Plants grown without shade tend to have higher shoot dry weights and dry matter than shaded plants. The present field study validates the light intensity effects observed in greenhouse-grown 'Tendergreen' and 'Florida Broadleaf' mustard greens (Makus and Lester, 2002). In that study, a 50% reduction in ambient light throughout the growing season resulted in reduced leaf ascorbate but higher leaf area, chlorophyll, carotenoids, total-N, NO_3-N, and most leaf mineral nutrients compared to plants grown in full light. In addition to light intensity, time of day appears to have a transient, but real effect, on leaf ascorbate and NO_3 levels, and pigment ratios.

In both the above snap bean and mustard greens studies, when leaf greenness or estimates of chlorophyll content were made using SPAD measurements with a Minolta 502 Chlorophyll Meter, leaf chlorophyll, which is estimated on an area basis, was higher in leaves grown at higher light levels when compared to leaves exposed to lower light levels. When leaf chlorophyll concentration is determined by solvent extraction and expressed on a dry wt. basis, the chlorophyll content is higher in shaded leaves. Plants compensate for reduced light environments by increasing leaf area. Because shading can reduce leaf temperatures, transpiration is reduced and leaves can maintain a higher turgor pressure and relative water content. Leaf dry matter is generally reduced by lower light levels, so that expressing leaf pigment content on a dry wt. basis can give higher values than when pigments are expressed on a unit area basis. In the case of the mustard greens grown in this study, there were no apparent visual differences between treatments in leaf greenness.

Our observations of effects of light intensity on ascorbate levels in *Brassica juncea* leaves were consistent with those in other plants. Mozafar (1994), in a review of the literature, concluded that light duration and/or intensity can increase ascorbate and that supplemental or adequate soil levels of K, Mn, Mo, Cu, Zn, and Co tend to improve ascorbate levels, whereas N application tends to improve carotene, niacin, riboflavin, and thiamine levels in vegetables.

Leaf ascorbate, chlorophyll, chlorophyll a:b ratios, and NO_3 levels were all affected by time of sampling. This finding has implications for reported levels of these compounds in the literature. Our data indicate that sampling for analysis of these compounds should be completed within as short time frame as possible and that the environmental conditions during sample collection, such as light, temperature, and vapor pressure or relative humidity, and time of day should be reported when presenting such data. The results suggest that vitamin C content in leafy mustard greens could be enhanced by harvesting during periods of the day characterized by high light intensity. However, such periods also often coincide with periods of higher temperatures and higher evaporative demand which can result in increased water loss and a reduction in produce quality especially for leafy vegetables that are sold on a fresh weight or water content basis.

CONCLUSIONS

Cloudy weather prior to harvest reduced leaf Vitamin C but elevated leaf greenness and many mineral nutrients. Time of day also influenced leaf ascorbate, nitrate, and chlorophylls. Researchers should be aware of these subtle changes, particularly if they are evaluating treatments where similar responses are being measured and require sampling over multiple time periods and/or over the course of the day. Producers and processors can maximize Vitamin C leaf content by harvesting mustard greens during periods of high light intensity.

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