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Spinach and Mustard Greens Response to Soil Texture, Sulfur Addition and Lithium Level

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ABSTRACT

A greenhouse experiment was conducted near Weslaco, Texas (Lat. 26° 8' N, Long. 97° 57' W) between December, 2006 and February, 2007 to evaluate the effect of soil texture, added sulfur and lithium level on the growth and leaf nutrients, particularly biofortified levels of leaf Li and S, in spinach and mustard greens. Cultivars Samish (*Spinacia oleracea* L.) and Florida Broadleaf [*Brassica juncea* (L). Czern.] were sown in sandy and silt loam soils. Spinach and mustard greens exhibited differences in most attributes tested. Mustard greens (MG) grown in the sandy loam soil produced plants with lower leaf and root dry wt., higher leaf transpiration and blade P, Mg, S, Li:K ratio, Zn, B and shoot and root Li. Spinach grown in the sandy loam soil had a higher shoot:root dry wt. ratio and blade K, Ca, Mg and Li:K ratio. Sulfur addition (1.1 t / ha equivalent) had no affect on agronomic responses measured, but reduced leaf blade K (spinach, only), Ca (spinach, only) and increased P (MG, only) and S. The micronutrients Mn, Zn, Cu and B were reduced in spinach and Na, Zn and Cu increased in MG by sulfur addition. In MG (only), adding Li from 0 to 40 kg / ha reduced plant wt., leaf number, area, fresh wt., root dry wt., and the incidence of powdery mildew and increased leaf transpiration. In MG, increasing soil applied Li increased leaf blade N, K, P, Mg, S, Fe and Zn. In spinach, Li addition decreased leaf blade K, P, S, and B. Added soil Li increased shoot and root Li in both greens. All reported increases or decreases due to Li addition over the 0 to 44 kg Li / ha range was linear. The Li shoot to root ratio and shoot level was higher in MG. Leaf blade S was reduced (r = -0.441**) by the presence of blade Li in spinach, but increased in MG (r = 0.496**). The ratio of leaf blade Li:K was approximately 4 X higher in MG, which may have interfered with stomatal regulation (higher leaf transpiration in MG with increasing Li applied).

Additional Index Words: Brassica juncea, *Spinacia oleracea*, leafy greens

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Leafy greens are an excellent source of mineral nutrients and vitamins for the human diet (U. S. Dept. Agriculture, 1982). The growing environment and cultural practices can affect the accumulation of nutrients in (leafy) vegetables. Mustard greens leaf nutrients and plant pigments are affected by soil texture, light intensity and cultivar (Makus and Lester, 2002); and in the case of leaf blade NO_3 and ascorbate levels, the time of harvest during the day is important (Makus and Lester, 2004).

Lithium salts applied to cucumbers can reduce the incidence of powdery mildew (Abood and Losel, 1991). Applied to potatoes, lithium inhibited the winged form of aphids (Harrewijn, 1983). Soil lithium content is quit variable, ranging from trace amounts to over 100 ppm (Kabata-Pendias and Pendias, 1984). In the Jordan Valley (Jordan), where soils ranged from 10 to 45 ppm Li, fruits from *Citrus limon*, *C. mitis*, and *C. paradisi* ranged from 14 to 70 ug/g Li, whereas Solanaceous plants such as pepper were reported to range from 194 to 318 ug /g Li in leaves (Haddadin et al., 2002). Watercress, grown hydroponically in 2 mM Li, accumulated 1216 ppm Li although growth was depressed at this solution concentration (Magalhaes et al., 1990). Plants have no Li requirement, but 1 mg / day has been proposed for humans weighing 70 kg. Vegetables, and in some cases drinking water, constitute 66 to 90% of the major dietary sources of Li, a mineral which has been implicated in human wellness (hypertension, depressive state, hormonal balance, leukemia, diabetes, dental carries, and immunological functions; Schrauzer, 2002; Magalhaes et al., 1990).

Soils used in vegetable production in south Texas vary in chemical and physical properties. These differences influence fertilizer and soil moisture dynamics and ultimately the nutritional quality of the vegetable crops grown on them. Furthermore, spring and fall-grown crops are often exposed to different temperature and light environments.

The objective of this greenhouse study was to evaluate the effects of soil texture, sulfur addition and lithium levels on two greens crops grown locally and to measure subsequent plant response and mineral leaf constituents.

MATERIAL AND METHODS

The experiment consisted of growing two leafy greens in two soils, with and without added sulfur at three levels of soil lithium, with all factorial treatments occurring once in each of four replicates in a completely random design. There were 96 pots in the experiment, each containing one plant. Two kg of air-dried, unsterilized sandy and silt loam soils were added separately to 15 cm dia. x 15 cm h white plastic pots and placed in a plastic-covered greenhouse receiving ambient (available) light. Minimum night temperatures were set at 12 ± 2 ºC and day temperatures were set to cool at 27 ± 2 ºC. Air temperature and photosynthetically active radiation (PAR) were measured every 60 s by a shielded thermocouple and a LI-190SA quantum sensor, respectively, and outputted hourly to a CR-10 data logger (Campbell Scientific, Logan, UT). Average hourly temperature and cumulative hourly PAR values were recorded over the respective growing periods. Five seeds per pot of spinach cultivar Samish and mustard greens cultivar Florida Broadleaf (Willhite Seed, Poolville, TX) were sown on Dec. 29, 2006. Plants were thinned to 1 per pot 14 days after planting (DAP). Water soluble 20N-20P-20K fertilizer at 250 mg per pot was added to pot saucers at 0 and 53 DAP, and 295 mg calcium nitrate per pot added at 39 and 60 DAP. Gypsum ($CaSO_4$) was incorporated into the soils of the amended sulfur treatment at 0.91 g / kg prior to filling the pots, to give a field equivalent rate of 1.1 t /ha. Lithium (as LiCl) was added at 0, 28, and 42 DAP for a total soil applied equivalent of 0, 11 and 44 kg Li/ha, respectively. All fertilizations and supplemental watering was applied to the pot saucers using deionized water. Frequency of watering was based on incipient visual soil drying. Minolta Chlorophyll Meter (Model SPAD-502) readings were taken at 61 (spinach and mustard greens) and 68 DAP (mustard greens, only).

Porometry measurements were made near solar noon on one representative fully expanded leaf one day before spinach (62 DAP) and mustard greens (68 DAP) harvests with a LI-1600 Steady State Porometer (LI-COR, Lincoln, NE). On each respective harvest date, plant fresh weight, leaf number, and leaf area (LI-1800 leaf area meter) were determined. At 62 DAP, mustard greens (only) were evaluated for powder mildew incidence (absent = 0, present = 1) and then infected plants were sprayed with Oxidate (BioSafe Systems, E. Hartford, CT) for subsequent control. The 0 and 1 ratings were converted to 0.01 and 10 for statistical analysis. After harvest, pots were stored at 10 ^{o}C until roots could be washed from the soil and then roots were forced-air dried at 60 ^{o}C for 3 days before weighing. Approximately 50 g of leaves were frozen, lyophilized, and ground by a Wiley Mill to pass through a 40 mesh screen (2 mm^2). From the latter sub-samples, pigments were analyzed spectrometrically (Wellburn and Lichtenthaler, 1984). Leaf and root (Li, only) nutrients were determined by ICP spectroscopy (Plank, 1992). Total leaf N and C was determined by dry combustion (Elementar Vario Max, Mt. Laurel, NJ).

Greens type, soil texture, sulfur addition, and lithium levels were designed and analyzed as a 2 x 2 x 2 x 3 factorial with 4 replications. All factorial treatments were arranged as a completely random design. 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, NC). Type I (sequential) sums of squares from PROC GLM were used to determine the polynomial fits.

RESULTS

Because of the large differences between greens and the many greens x main effect interactions, the analysis was reduced to a 2 x 2 x 3 and comparisons made within each greens type. Interactions, many of which were slope or magnitude differences, will be discussed, but interaction means will not be shown.

Soils and environment. The characteristics of the two soils have been reported elsewhere (Makus and Lester, 2002). Basically, the sandy loam textured soil contained the least amount of clay (11 vs. 23%) and carbon (0.30 vs. 1.01%), and has lower pH (7.6 vs. 8.0) and electrolytes (0.08 vs. 0.68 dS/M) and higher bulk density (1.51 vs. 1.22) compared to the silt loam soil. The silt loam soil was typically 2 to 18 times higher in extractable Ca, K, Mg, Mn, P, S, Na, Zn, Cu, and B than the sandy loam soil. Lithium soil test values were 1.7 and 4.3 ppm for the sandy and silt loam soils, respectively.

Mean temperatures and cumulative average hourly photon flux (as PAR) between seed sowing and spinach harvest (62 days later) were 18.08 ^{o}C and 222 mmol/s/m^2 and for mustard greens, 18.12 ^{o}C and 250 mmol/s/m^2, respectively.

There were significant differences between greens type in most attributes, to include spinach and mustard greens interactions with soil texture, sulfur addition, and lithium level. Therefore results will be summarized by greens type. Leaf pigments (data not shown) were not affected by any soil or Li level, with the exception of an 11% reduction in total leaf blade chlorophyll when sulfur was added to soil containing mustard greens (P<0.038).

Spinach.

Agronomic. 'Samish' leaf number and shoot:root ratio was higher when grown in sandy loam soil, but leaf area (P<0.06) was higher in plants grown in silt loam soil (Table 1.). Neither sulfur addition, as $CaSO_4$, nor added LiCl had an affect on any of the agronomic responses measured.

Leaf Total N, C and Mineral macro-nutrients. 'Samish' grown in sandy loam soil had higher leaf blade K, Ca, Mg, total cations and a Li:K ratio compared to silt loam-grown 'Samish' (Table 3.). The total N and C levels were higher in silt loam-grown 'Samish'. Adding no sulfur resulted in higher K, Ca and total cations (sandy loam only) compared to plants that received gypsum. Total S leaf blade level was higher when sulfur was added to the silt loam soil. Leaf blade levels of K (sandy loam only), P, S and total cations all decreased with increasing application rates of applied Li, but the Li:K ratio increased (all polynomial fits were linear, L).

Mineral micro-nutrients. The Na and Mn leaf blade levels were higher in silt loam-grown spinach (Table 4.). Zinc, B, shoot and root Li and the Li shoot:root ratio was higher in sandy loam-grown spinach. Adding no sulfur resulted in increased leaf blade Mn, Zn, Cu and B (sandy loam only) compared to adding sulfur fertilizer. The addition of increasing rates of Li increased shoot and root Li (L), Li shoot:root ratio (L,Q) and decreased leaf blade B (sandy loam only).

Mustard greens.

Agronomic. 'Florida Broadleaf' grew better in the silt loam soil. Although leaf number, shoot:root ratio and mildew index were not affected, other attributes measured were higher than in plants grown in the sandy loam soil (Table 1.). Sulfur addition had no affect on any of the attributes measured. Increasing Li application rate linearly decreased plant wt., leaf number and area, leaf fresh wt., root dry wt. and the incidence of leaf mildew. Neither leaf dry wt. nor shoot:root ratio were affected by Li application.

Leaf porometry and greenness. Mustard greens grown in silt loam soil had lower transpiration rates or greater diffusive resistance and higher leaf temperature than did plants grown in sandy loam soil (Table 2.). Sulfur addition increased diffusive resistance and leaf temperature. Increasing Li application rate increased leaf transpiration (L), decreased diffusive resistance (L,Q), and increased leaf greenness or SPAD values (L).

Leaf Total N, C and Mineral nutrients. 'Florida Broadleaf' plants grown in sandy loam soil had higher P, Mg, S, total cations and a Li:K ratio than did silt loam-grown plants (Table 3.). Only Ca was higher in plants grown in the silt loam soil. Added sulfur improved P and S, but reduced the Li:K ratio in leaf blades. Increasing the Li rate increased N, K, P, Mg, S, total cations (all L) and the Li:K ratio (L, Q).

Mineral micro-nutrients. Growing 'Florida Broadleaf' in sandy loam soil improved leaf blade Mn, Zn, B and shoot and root Li levels (Table 4.). Lithium levels and Fe levels were higher in silt loam-grown plants. Adding no sulfur resulted in higher leaf blade Na, whereas supplemental sulfur increased leaf blade Zn and Cu levels. Increasing Li rates increased leaf blade Fe, Zn, shoot and root Li (all L) and the Li shoot:root ratio (L, Q).

Linear correlations. Leaf total sulfur was the only mineral nutrient found to be correlated with leaf Li (r = -0.441, P<0.01 in 'Samish'; and r = 0.496, P<0.01 in 'Florida Broadleaf'). Shoot and root Li levels were correlated 0.883 (P<0.01) and 0.924 (P<0.01) in spinach and mustard greens, respectively. In spinach, leaf blade Li was correlated negatively with K (-0.285*), P (-0.474**), and Mn (-0.323*). Leaf blade S was correlated positively with K (0.598**), Mg (0.343*), Na (0.392**), and total cations (0.559**). In mustard greens, leaf blade Li was correlated positively with K (0.498**), Ca (0.337*), P (0.821**), Mg (0.729**), Fe (0.321*), Zn (0.444*), and total cations (0.564**) and leaf blade S with K (0.595**), P (0.610**), Mg (520**), Fe (0.350**), Zn (0.492**) and total cations (0.527**).

DISCUSSION

In a previous study, when mustard green cultivars Florida Broadleaf and Tendergreen were grown in light and heavy textured soils, heavier textured soils improved most leaf nutrient concentrations except P and Fe (Makus and Lester, 2002). In the present study, using two of these soil types, total leaf blade cations were actually less when grown in the heavier silt loam soil. 'Samish' leaves were twice as high as 'Florida Broadleaf' leaves in total cations when grown in both soil textures, suggesting that 'Samish' leaves

Table 1. Agronomic response of spinach and mustard greens grown in two soil textures, with and without added sulfur and at three levels of soil lithium.[z]

Main effects	Plant WT. (g)	Leaf number	Area/leaf (cm^2)	Leaf fresh WT. (g)	Leaf dry WT. (g)	Root dry WT. (g)	Shoot:Root DW ratio	Mildew index[y]
	- Mustard greens -							
Soil texture:								
Sandy loam	53.6b	8.25a	82.5b	47.1b	16.7b	2.70b	6.39a	2.1a
Silt loam	62.0a	8.50a	96.4a	55.7a	19.9a	3.04a	6.97a	3.3a
	**[x]	NS	**	**	**	*	NS	NS
Sulfur:								
No	57.1a	8.17a	90.2a	50.3a	17.7a	2.74a	6.87a	2.1a
Yes	58.4a	8.58a	88.6a	52.4a	18.8a	3.00a	6.48a	3.3a
	NS	NS	NS	NS	NS	NS	NS	NS
Lithium level (LL):								
0 kg/Ha	63.2a	8.88a	102a	55.2a	17.6a	3.15a	5.80a	5.0a
11 kg/Ha	59.5a	8.50a	92.0a	53.0a	19.6a	3.01a	7.00a	3.1ab
44 kg/Ha	50.6b	7.75b	74.2c	45.9b	17.5a	2.45b	7.24a	0.0b
	**	**	**	**	NS	**	NS	**
Polynomial fit for LL:	L**	L**	L**	L**	---	L**	$Q^{0.10}$	L**
	- Spinach -							
Soil texture:								
Sandy loam	31.9a	12.9a	35.6b	23.5a	3.83a	2.19a	1.83a	
Silt loam	32.8a	11.4b	39.3a	22.1a	3.58a	2.26a	1.59b	
	NS	*	0.06[w]	NS	NS	NS	**	
Sulfur:								
No	32.9a	12.2a	37.3a	22.6a	3.67a	2.29a	1.65a	
Yes	31.8a	12.2a	37.6a	23.0a	3.75a	2.16a	1.78a	
	NS	NS	NS	NS	NS	NS	NS	
Lithium level (LL):								
0 kg/Ha	32.6a	12.4a	37.1a	23.7a	3.71a	2.14a	1.74a	
11 kg/Ha	32.7a	11.7a	38.5a	21.8a	3.60a	2.28a	1.63a	
44 kg/Ha	31.8a	12.4a	36.8a	22.9a	3.82a	2.24a	1.76a	
	NS	NS	NS	NS	NS	NS	NS	
Polynomial fit for LL:	—	—	—	—	—	—	—	

[Z] There were no interactions ($P < 0.05$).
[Y] Mustard greens only.
[X] NS, ** and * = not significant or significant at $P < 0.01$ and $P < 0.05$, respectively. L = Linear, Q=quadratic.
[W] Prob. > 'F' value.

Table 2. Effect of soil texture, sulfur application and lithium level on mustard greens leaf transpiration, diffusive resistance, temperature and greenness (Minolta SPAD values) measured on March 7, 2007.[Z]

Main Effects	Transpiration ug/cm^2/s	Diffusive resistance s/cm	Leaf temperature ^{o}C	SPAD value
Soil texture:				
Sandy loam	6.78 a	3.48 b	28.1 b	26.0 a
Silt loam	5.00 b	4.61 a	28.7 a	26.3 a
	*[Y]	*	**	NS
Sulfur:				
No	6.35 a	3.48 b	28.2 b	26.0 a
Yes	5.42 a	4.60 a	28.6 a	26.2 a
	NS	*	*	NS
Lithium level (LL):				
0 kg/ha	5.12 b	3.96 ab	28.4 a	24.6 b
11 kg/ha	5.34 b	5.17 a	28.5 a	24.9 b
44 kg/ha	7.20 a	2.99 b	28.2 a	28.9 a
	0.06[X]	**	NS	**
Polynomial fit for LL:	L**	L*, Q**	--	L**

[Z] There were no interactions.
[Y] NS, *, ** = not significant or significant at P = 0.05 and P = 0.01, respectively.
Means separated by different letters are significant at the prob. level shown.
[X] Prob. of a greater 'F' value.

were more nutrient dense compared to those of mustard greens.

In this study, we report sulfur as total S without regard to origin as a salt or organic form. It should be noted that plant organic sulfur compounds are associated with human wellness, several of which have been reported in spinach (Demirkol et al., 2004). Organic sulfur components in plants consist of such compounds as the sulfur containing primary amino acids, taurine, and glutathione. Sulfur transfer compounds such as Coenzyme A, S-adenyl-methionine, S-methyl methionine and dimethyl sulfonic hydroxbutyrate are needed to produce thiols, thioethers, sulfoxides, methyl-sulfonium compounds, sulfate esters (polysaccharide sulfates), sulfamates (mustard oil glycosides) and sulfonic acids. When consumed, S-compounds such as methyl sulfonylmethionine, supply S-compounds for joint function. Low dietary levels of another S-compound, N-acetyl-cysteine, have been implicated in Parkinson's disease.

Soil test S levels reported for the sandy loam soil were one-twentieth the levels found in the silt loam soil (Makus and Lester, 2002). However, supplemental S application improved leaf blade S in spinach by 10% and in mustard greens by 54% in the silt loam soil compared to the sandy loam soil. 'Florida Broadleaf' leaf blades had almost twice the leaf S content as did 'Samish' and the increase in 'Florida Broadleaf' was linear whereas 'Samish' leaf blade S level decreased with increasing Li application rates. Lithium, which is reported to suppress Ca uptake in bush beans (Wallace and Shear, 1979), had no affect in this study, but, in a previous study, Li application at 10 kg/ha reduced Ca levels in 'Florida Broadleaf' but not in 'Samish' leaves (Makus and

Table 3. Effect of soil texture, sulfur addition and lithium level on leaf macro-nutrients in spinach and mustard greens.[Z]

Main Effects	N	C	K	Ca	P	Mg	S	Total cations	Li:K ratio
	- - - - - - - - - -	- - - - - - - - - -	- - - - - - %	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - -	$x10^4$
					Spinach				
Soil texture:									
Sandy loam	1.80 b	36.2 b	4.97 a	1.71 a	0.600 a	0.488 a	0.183 a	7.21 a	34 a
Silt loam	1.95 a	36.9 a	4.52 b	1.45 b	0.595 a	0.419 b	0.189 a	6.42 b	17 b
	*[Y]	**	**	**	NS	**	NS	**	**
Sulfur:									
No	1.88 a	36.4 a	4.95 a	1.66 a	0.624 a	0.469 a	0.178 b	7.11 a	26 a
Yes	1.88 a	36.7 a	4.55 b	1.50 b	0.570 a	0.438 a	0.194 a	6.52 b	25 a
	NS	NS	*	*	NS	NS	**	**	NS
Lithium level:									
0 kg/ha	1.93 a	36.5 a	5.12 a	1.57 a	0.616 a	0.457 a	0.194 a	7.18 a	<1 c
11 kg/ha	1.82 a	36.7 a	4.66 b	1.60 a	0.633 a	0.431 a	0.189 ab	6.72 ab	15 b
44 kg/ha	1.88 a	36.4 a	4.46 b	1.56 a	0.544 b	0.473 a	0.176 b	6.54 b	61 a
	NS	NS	**	NS	*	NS	*	*	**
Polynomial fit:	--	--	L**	--	L*	--	L*	L*	L**
Significant interactions:									
Soil x sulfur		*	**			**	**	**	**
Soil x Li			**		*			**	**
Sulfur x Li			*					*	
	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	*Mustard greens*	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	
Soil texture:									
Sandy loam	1.05 a	37.8 a	1.70 a	1.28 b	0.358 a	0.144 a	0.320 a	3.49 a	125 a
Silt loam	1.02 a	37.9 a	1.67 a	1.61 a	0.297 b	0.118 b	0.301 b	3.09 b	76 b
	NS	NS	NS	**	**	**	*	**	**
Sulfur:									
No	1.04 a	37.8 a	1.64 a	1.45 a	0.318 b	0.129 a	0.244 b	3.24 a	107 a
Yes	1.04 a	37.8 a	1.74 a	1.44 a	0.337 a	0.132 a	0.377 a	3.34 a	94 b
	NS	NS	NS	NS	*	NS	**	NS	*
Lithium level:									
0 kg/ha	0.85 c	38.1 a	1.61 b	1.43 a	0.274 c	0.119 b	0.266 c	3.16 b	1 c
11 kg/ha	0.99 b	37.9 ab	1.61 b	1.39 a	0.317 b	0.124 b	0.295 b	3.14 b	75 b
44 kg/ha	1.27 a	37.6 b	1.84 a	1.52 a	0.390 a	0.149 a	0.370 a	3.56 a	227 a
	**	**	**	NS	**	**	**	**	**
Polynomial fit:	L**	L**	L**	--	L**	L**	L**	L**	L**,Q**
Significant interactions:									
Soil x sulfur							*		**
Soil x Li	**				**				**
Sulfur x Li							**		

[Z] There were no third order interactions.
[Y] NS, **, * = not significant or significant at $P < 0.01$ and $P < 0.05$, respectively. L = linear, Q = Quadratic.

Table 4. Effect of soil texture, sulfur addition and lithium level on leaf micro-nutrients in spinach and mustard greens.

Main Effects	Na	Mn	Fe	Zn	Cu	B	Shoot Li	Root Li	Shoot:root ratio
	μg/g								
	Spinach								
Soil Texture:									
Sandy loam	476 b	59.4 b	44.7 a	124 a	4.67 a	76.8 a	150 a	146 a	0.759 a
Silt loam	781 a	91.3 a	43.5 a	90 b	4.50 a	65.2 b	71 b	114 b	0.536 a
	**[z]	**	NS	**	NS	**	**	**	NS
Sulfur:									
No	592 a	81.0 a	43.3 a	111 a	5.08 a	74.7 a	113 a	134 a	0.721 a
Yes	665 a	69.9 b	44.9 a	103 a	4.08 b	67.3 b	108 a	126 a	0.574 a
	NS	*	NS	**	**	**	NS	NS	NS
Lithium level:									
0 kg/ha	622 a	71.7 a	47.6 a	116 a	4.75 a	77.1 a	<1 c	2 c	0.26 b
11 kg/ha	634 a	79.5 a	43.1 a	104 a	4.44 a	71.1 ab	65 b	83 b	0.82 a
44 kg/ha	629 a	75.1 a	41.6 a	102 a	4.56 a	64.9 b	266 a	305 a	0.86 a
	NS	NS	NS	NS	NS	**	**	**	**
Polynomial fit:	--	--	--	--	--	--	L**	L**	L**, Q**
Significant interactions:									
Soil x sulfur	**				**	**		*	
Sulfur x Li						**		**	
Soil x sulfur x Li					*				
	Mustard greens								
Soil texture:									
Sandy loam	261 a	23.8 b	25.1 b	24.2 a	3.17 a	43.5 a	226 a	27 a	6.82 a
Silt loam	244 a	28.4 a	28.5 a	16.3 b	2.83 a	38.5 b	131 b	17 b	6.81 a
	NS	*	*	**	NS	**	**	**	NS
Sulfur:									
No	234 a	26.4 a	25.8 a	18.9 b	2.54 b	40.0 a	183 a	25 a	6.41 a
Yes	270 b	25.8 a	27.8 a	21.6 a	3.46 a	42.0 a	174 a	18 b	7.21 a
	*	NS	NS	**	*	NS	NS	**	NS
Lithium level:									
0 kg/ha	260 a	26.7 a	25.2 b	19.4 b	3.06 a	38.9 a	1 c	1 c	0.95 b
11 kg/ha	251 a	26.0 a	24.4 b	18.9 b	2.69 a	42.6 a	120 b	14 b	10.83 a
44 kg/ha	247 a	25.6 a	30.8 a	22.4 a	3.25 a	41.5 a	414 a	50 a	8.66 a
	NS	NS	**	**	NS	NS	**	**	**
Polynomial fit:	--	--	L**	L**	--	--	L**	L**	L**, Q**
Significant interactions:									
Soil x sulfur			**				**		
Soil x Li					**				

[Z] NS, **, * = not significant or significant at $P < 0.01$ and $P < 0.05$, respectively. L=linear; Q=quadratic.

Lester, 2002). Therefore, species differences appear to be important in the way lithium addition affects both S and Ca uptake.

In a field experiment in soil ranging from 1.4 to 2.4 ppm Li, addition of 1 kg Li / ha increased potato tuber citric and malic acids (Vlasyuk et al., 1973). In a hydroponic experiment, solution Li concentrations to 12 ppm increased cucumber fruit yield, fruit sugar, and ascorbic acid, but Li did not accumulate in the fruit (Rusin, 1979). Tomato and bush bean plants grown in solutions up to 0.5 mM Li accumulated Li in roots, leaves and fruits/pods; yields were reduced by Li solution levels greater than 0.1 mM (Patel, et al., 1980). In the present study, the greatest leaf Li concentrations were found in mustard greens grown in the sandy loam soil (530 µg/g). When the highest Li rates were applied, leaves of mustard greens appeared somewhat crinkled or deformed and exhibited necrotic leaf margins. The marginal leaf necrosis, owing possibly to desiccation, a high Li:K ratio (stomatal control), greater transpiration rates, and higher plant shoot to root ratios at increasing levels of applied Li, would suggest that high levels of lithium salts in the soil may decrease water use efficiency in mustard greens. The highest Li rate increased SPAD leaf greenness readings (Table 2), but not spectrometrically determined leaf chlorophyll based upon leaf dry weight (data not shown). High soil Li rates decreased leaf area and fresh weight, but not dry weight. Since SPAD measurements are based on area, and not dry weight, the high leaf SPAD value for the highest soil Li rate appears to be misleading.

In cucumbers, the expression of powdery mildew (*Podosphaera xanthii*, formerly *Sphaerotheca fuliginea*) was reversed in the presence of LiCl (Losel et al, 1994). The authors suggested that this might be due to interference with phosphatidyl inositol turnover which was higher in infected control plants. Ultrastructure studies by Baka et al. (2002) revealed that high LiCl levels (9 vs. 3 mM or less) resulted in decreased host leaf chlorophyll level and pronounced cellular disorganization in *Vicia faba* L. when Li was used to control of *Botrytis fabae* Sard.

CONCLUSIONS

Mustard greens appear to respond favorably to the addition of S and Li with respect to leaf blade nutrients when compared to spinach. Mustard greens biomass was reduced by Li application, with leaves showing some distortion and marginal necrosis at the highest rate; but the incidence of leaf powdery mildew was eliminated by the 44 kg/ha Li rate. Mustard greens transported or took up more Li by the roots and transported Li to the shoots than did spinach (r = 0.924** vs. r = 0.883**). The higher leaf blade Li in mustard greens resulted in higher levels of leaf blade S (r = 0.496**) whereas higher leaf blade Li in spinach reduced leaf blade S (r = -0.441 **). The Li:K ratio was about 4 X higher in mustard greens compared to spinach, which may have affected stomatal regulation, resulting in increased leaf respiration rates and subsequent leaf marginal necrosis in 'Florida Broadleaf'. Response of spinach, generally, to increasing Li application resulted in similar or reduced levels of nutrients with increasing Li rates and virtually no agronomic benefit, whereas the Li affect on mustard greens nutrients was the opposite, indicating that greens type vary considerably in their response to added Li. The addition of soil applied Li for the purpose of biofortification was improved in sandy-loam compared to silt loam soils with leaf blade levels averaging around 266 (spinach) to 414 (mustard greens) ug/g dry weight at the highest application rate. Addition of sulfur fertilizer, as gypsum, improved leaf blade S levels from 9 (spinach) to 54 % (mustard greens).

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