Influence of Sublethal Glyphosate Rates on Leaf Mineral Concentration of Tomato

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Abstract. Glyphosate at sublethal rates was applied prebloom, at-bloom, or postbloom relative to the first flower cluster to tomato (*Lycopersicon esculentum* Mill.) to determine the effect on foliar concentrations of N, P, K, Ca, and Mg. Glyphosate rates of 0, 1, 6, 10, 60, and 100 g·ha⁻¹ were used to simulate the effects of spray drift. In three studies, plant vigor declined with increased glyphosate rates and younger plants were more sensitive than older plants. Plant height decreased as glyphosate rate increased, but the response differed with time of evaluation and with stage of development. In Expt. 1, N content decreased with increasing rate of glyphosate, regardless of stage of development, but response varied with time of evaluation with prebloom and at-bloom applications. In Expt. 2, prebloom glyphosate applications reduced N content, but applications at-bloom did not. P declined with prebloom and at-bloom glyphosate applications in Expt. 1, but only with prebloom applications in Expt. 2. In Expt. 3, P concentrations generally declined with glyphosate rates ≤10 g·ha⁻¹, but were unchanged or increased with rates of 60 and 100 g·ha⁻¹. Tissue K, Ca, and Mg concentrations were not consistently affected by glyphosate rate and sample times. Although significant changes in foliar concentrations of N, P, K, Ca, and Mg occurred, leaf mineral analysis was not considered to be a reliable method of quantifying sublethal effects of glyphosate in tomato. Mineral deficiency did not occur in response to glyphosate application. Chemical name used: N-(phosphonomethyl)glycine (glyphosate).

Glyphosate is a broad-spectrum, nonselective, postemergence herbicide with activity against most annual and perennial plants (Franz, 1985). Its primary mechanism of action is the inhibition of the biosynthesis of aromatic amino acids (Cole, 1985). Its effectiveness has led to widespread use, which is expected to increase with the continued development and adoption of transgenic crops with resistance to glyphosate (Powles et al., 1998). Increased usage will present greater opportunities for the occurrence of spray drift and injury to susceptible nontarget crops. In Florida, a variety of horticultural crops are often grown in close proximity. Glyphosate is used for weed control prior to land preparation for a new crop, for perimeter and ditch bank weed control of established crops, and for weed management in perennial crops such as citrus. Spray drift in such situations poses a problem to adjacent crops, the extent of

which bears further investigation. Simulated drift studies have been conducted to evaluate the effect of sublethal rates of glyphosate on the yield of processing tomato (Romanowski, 1980). Total yields were reduced by 100 g·ha⁻¹ glyphosate applied in early or mid-season; however, yield was unaffected by late-season exposure.

Although the effect of sublethal glyphosate rates on foliar mineral content of tomato has not been previously reported, investigations of glyphosate effects on ion uptake and translocation have been conducted with 'Eastern Butterwax' bean (*Phaseolus vulgaris* L.) and 'Hill' soybean [*Glycine max* (L.) Merr.]. Brecke and Duke (1980) found that glyphosate inhibited the absorption of ⁸⁶Rb and ³²P by bean leaf cells. Duke et al. (1983) reported that when roots of soybean seedlings were exposed to glyphosate, uptake of Ca was unaffected, but the translocation of Ca to leaves and cotyledons was significantly reduced. They suggested that this could partially account for severe retardation of leaf growth by glyphosate. In subsequent studies, the effects of glyphosate on Ca were compared with those on Mg and K. Uptake and translocation of Ca and Mg, but not of K, were reduced by foliar or root-applied glyphosate (Duke et al., 1985). The objectives of the present studies were to evaluate the effects of sublethal rates of glyphosate

applied at three stages of development on vigor, height, and foliar N, P, K, Ca, and Mg concentrations of fresh-market tomato plants. Characteristic changes in mineral composition may aid in diagnosing glyphosate drift injury, and adjusting nutrition to ameliorate the inhibition of growth and yield that occurs with nonlethal exposure to glyphosate.

Materials and Methods

The effect of glyphosate rate on foliar mineral content was assessed in three experiments conducted at Bradenton, Fla. The experimental designs were randomized complete blocks with five replications in Expt. 1 and six replications in Expts. 2 and 3. Fifteen-cm high raised beds were prepared, fertilized with 336 kg·ha⁻¹ of N and 465 kg·ha⁻¹ K from an 18N-0P-30K formulation applied in two bands, fumigated with 392 kg·ha⁻¹ of a 67% methyl bromide + 33% chloropicrin mixture, and covered with polyethylene mulch. The experiments were conducted on an Eau Gallie fine sand (sandy, siliceous, hyperthermic, Alfic Haplaquod), which was subsurface-irrigated. Tomato plants, cultivar Sunny, were transplanted on 23 Feb. 1988, 13 Sept. 1988, and 17 Feb. 1989. In each experiment, glyphosate (Roundup 4E®) was applied at three stages of development at 0, 1, 10, and 100 g·ha⁻¹. Because significant responses were obtained with 10 and 100 g·ha⁻¹ glyphosate in Expts. 1 and 2, additional rates of 6 and 60 g·ha⁻¹ were included in Expt. 3. Prebloom (for first cluster) sprays were applied at 4 weeks after transplant (WAP) in Expts. 1 and 2, but at 3 WAP in Expt. 3. Glyphosate was applied at bloom 5 WAP and the postbloom applications were made 7 to 8 WAP.

In Expt. 1, eight plants per plot were spaced 0.46 m apart on 0.76-m-wide beds with an inter-row spacing of 1.37 m. In Expts. 2 and 3, ten and eight plants per plot, respectively, were spaced 0.61 m apart on 0.81-m-wide beds with a 1.52-m inter-row spacing. In Expts. 1, 2, and 3, nonplanted alleys at the end of each plot were 2.44, 1.52, and 1.22 m, respectively; and treatment rows were separated by a guard row in all experiments.

Plant vigor was assessed, plant height was measured, and the youngest mature leaf was sampled from each plant in the row for leaf nutrient analysis at 7, 14, 21, and 28 d after treatment (DAT). Plant vigor was evaluated visually using a 0 to 10 scale. A vigor rating of 0 indicated that all plants were dead, while 10 represented optimum plant growth. Leaf samples were dried at 70 °C and ground to pass a 40-mesh screen prior to determination of total N, P, K, Ca, and Mg concentrations. Tissue was digested by micro-Kjeldhal procedures and total N was determined by rapid-flow analysis (Hanlon et al., 1994). The concentrations of Ca, P, K, and Mg were determined from dry, ashed tissue by inductively coupled plasma emission spectrometry (Hanlon et al., 1994). Concentrations of N were determined for Expt. 1 and for prebloom and at-bloom applications of Expt. 2. Leaf N

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data were not available for Expt. 3 and for postbloom applications in Expt. 2.

Tests of significance for main effects of herbicide rate, stage of growth, sampling date, and interactions were conducted using analysis of variance (SAS Release 6.12). Orthogonal polynomials were used to evaluate the nature of the response to increased rates of glyphosate.

Results

Plant vigor and plant height. Plant vigor decreased as rate of glyphosate increased; however, the most pronounced suppression of vigor occurred with prebloom glyphosate applications (Table 1). More mature plants were less susceptible to the deleterious effects of sublethal rates of glyphosate. Within each stage of development, the suppression of plant vigor was progressive, with losses in vigor apparent by 7 DAT. Marked decline in vigor over time was observed with 60 and 100 g·ha⁻¹. The lowest plant vigor ratings occurred with the highest glyphosate rate at 28 DAT.

The effect of glyphosate rate and time of assessment after application on plant height did not differ with stage of development in Expt. 1; therefore, data for prebloom and at-bloom applications were averaged over stage of development (data not shown). Slowing of growth with 10 and 100 g·ha⁻¹ glyphosate resulted in a linear decline ($P \leq 0.01$) in plant height with increasing glyphosate rates by 7 DAT. Applications of 100 g·ha⁻¹ glyphosate slowed tomato growth to 2.5 cm over the 4-week evaluation period compared with 36.7 cm for nontreated plants. The profound slowing of growth with 100 g·ha⁻¹ glyphosate vs. continued growth with the other rates resulted in quadratic responses to increasing glyphosate rates at 14, 21, and 28 DAT ($P \leq 0.05$). In Expts. 2 and 3, the response to glyphosate rate and time of evaluation differed with the stage of development at application (Table 2). In Expt. 2, plant height, like plant vigor, was affected by glyphosate rate to a lesser degree when application occurred at bloom and postbloom than when applied prebloom. Plant height decreased linearly with increasing rates of glyphosate applied at all stages of development. Differences in height between nontreated plants and plants treated with 100 g·ha⁻¹ glyphosate increased with time of evaluation after prebloom and at-bloom applications.

The additional rate of 60 g·ha⁻¹ used in Expt. 3 significantly reduced plant height (Table 2). Decreases in plant height with increasing rates of glyphosate were linear or quadratic in nature and differed with time of evaluation and stage of development at application. The least growth inhibition occurred with plants treated postbloom. The effect of increased glyphosate rate on plant height became more pronounced with time, as growth continued in nontreated plants and plants treated with lower rates of glyphosate, whereas much less growth occurred in plants treated with 60 and 100 g·ha⁻¹ glyphosate.

Leaf mineral content. Expt. 1. Leaf N concentration varied with glyphosate rate, stage

Table 1. Effects of sublethal rates of glyphosate and stage of development on tomato plant vigor.^z

		Time of treatment											
Rate		Prebloom				At-bloom				Postbloom			
(g·ha ⁻¹)	DAT ^y :	7	14	21	28	7	14	21	28	7	14	21	28
<i>Expt. 1</i>													
0		9.9	9.6	9.4	9.6	10.0	10.0	10.0	8.9	9.7	9.2	9.7	9.2
1		9.3	9.2	9.4	8.8	9.1	9.0	8.9	8.7	9.0	9.4	9.4	8.1
10		8.0	5.4	6.3	6.8	8.5	8.0	7.3	8.1	9.3	9.2	9.0	8.2
100		4.7	3.2	2.9	1.1	6.3	3.8	3.1	2.1	8.5	7.2	6.1	5.9
Significance		Q**	Q**	Q**	Q**	Q**	Q**	Q**	L**	L**	L**	L**	L**
<i>Expt. 2</i>													
										Mean ^x			
0		9.8	9.2	9.2	9.4	8.6	8.9	9.0	8.8	9.1			
1		9.4	9.3	9.2	9.1	9.3	9.4	9.3	8.7	9.1			
10		9.2	7.8	8.3	8.3	8.6	9.1	9.1	8.9	8.6			
100		8.3	4.8	3.9	3.1	7.7	6.8	6.8	6.3	8.1			
Significance		L**	Q**	L**	L**	L**	L**	L**	L**	Q**			
<i>Expt. 3</i>													
0		9.4	9.3	9.2	8.9	9.3	9.4	9.2	8.8	9.5	9.5	9.1	9.3
1		9.3	8.7	8.5	8.2	9.2	9.0	9.0	8.6	9.2	9.1	8.7	8.8
6		9.2	8.6	8.0	8.2	9.2	9.0	8.6	8.6	8.9	8.8	8.7	8.4
10		9.1	7.7	7.8	8.3	9.4	8.4	8.5	8.4	8.7	8.6	8.5	8.0
60		8.8	4.0	4.2	3.9	8.6	5.8	5.4	4.4	8.7	7.6	7.3	6.7
100		8.1	3.0	2.3	1.7	8.8	5.4	5.1	3.4	8.5	7.3	6.6	6.3
Significance		L**	Q**	Q**	Q**	L**	Q**	Q**	Q**	L**	Q**	Q**	Q**

^zVigor was measured on a scale of 10 (100% vigor) to 0 (death).

^yDAT = days after treatment.

^xPlant vigor averaged over all evaluation times.

**Linear (L) and quadratic (Q) effects significant at $P \leq 0.01$.

Table 2. Effects of sublethal rates of glyphosate and stage of development on tomato plant height (cm).

		Time of treatment											
Rate		Prebloom				At-bloom				Postbloom			
(g·ha ⁻¹)	DAT ^z :	7	14	21	28	7	14	21	28	7	14	21	28
<i>Expt. 2</i>													
										Mean ^y			
0		54.5	74.0	88.7	100.0	83.0	99.0	105.8	114.2	116.1			
1		54.5	73.5	90.0	102.2	86.7	103.2	106.2	117.8	116.7			
10		54.7	69.7	85.3	100.0	86.7	100.3	104.8	114.8	113.2			
100		45.5	50.0	52.0	57.3	80.2	83.3	86.5	92.2	109.6			
Significance		L**	L**	L**	L**	L*	L**	L**	L**	L**			
<i>Expt. 3</i>													
0		---	26.5	36.7	54.2	46.2	67.7	78.7	93.0	80.7	96.2	101.0	107.0
1		---	25.5	35.8	52.3	45.7	65.7	78.2	91.0	78.3	93.0	99.8	103.0
6		---	27.0	36.3	52.8	44.3	66.0	77.3	92.0	78.5	92.5	99.3	102.8
10		---	25.0	35.3	48.2	45.3	65.3	75.3	90.3	76.0	91.3	96.3	101.7
60		---	19.0	22.8	33.5	41.3	54.5	60.0	66.2	74.2	86.2	86.5	89.7
100		---	14.3	14.5	15.7	43.7	51.5	53.2	54.5	73.2	80.5	80.3	80.8
Significance		---	L**	L**	L**	Q*	Q**	Q**	Q**	L**	L**	Q*	Q*

^zDAT = days after treatment.

^yPlant height averaged over all sampling times.

*Linear (L) and quadratic (Q) effects significant at $P \leq 0.05$ or 0.01.

of development, and time of evaluation after treatment ($P = 0.0003$). Increased rates of glyphosate applied prebloom and at-bloom reduced N concentration of tomato leaves, and the response varied with time after treatment (Table 3). Applications of glyphosate postbloom resulted in a quadratic decrease in leaf N concentrations from 5.3% (control) to 4.6% (100 g·ha⁻¹ glyphosate). Generally, glyphosate at 100 g·ha⁻¹ reduced N concentration.

The effect of glyphosate exposure at rates from 1 to 100 g·ha⁻¹ on leaf P, K, Ca, and Mg concentrations was dependent upon the stage of plant development at application ($P = 0.0001$). Leaf P concentration declined linearly with an increase in glyphosate rates applied at the prebloom and bloom stages, but P content was unaffected by glyphosate ap-

plied postbloom (Fig. 1A). Leaf K concentration decreased linearly with increased glyphosate rates, and the rate of decline was lowest when glyphosate was applied postbloom (Fig. 1B). Glyphosate applied prebloom and at bloom did not influence Ca concentration (Fig. 1C). However, Ca concentration increased linearly with increasing rates of glyphosate applied postbloom. Concentrations of Mg were highest with postbloom applications and increased as glyphosate rate was increased from 0 to 10 g·ha⁻¹ (Fig. 1D). No change in Mg concentration occurred with increased rates of glyphosate applied prebloom, but the concentration declined linearly with glyphosate applications at bloom.

Expt. 2. Foliar N data were available for four weekly evaluations after prebloom

Table 3. Effects of rate and time of application of glyphosate, and time of sampling, on N concentration (%) of tomato leaves (Expt. 1).

Rate (g·ha ⁻¹)	DAT ² :	Time of treatment							
		Prebloom				At-bloom			
		7	14	21	28	7	14	21	28
0		5.6	5.6	5.6	5.1	5.4	5.4	5.5	4.4
1		5.7	5.5	5.3	4.6	6.0	5.3	5.4	5.6
10		5.1	5.0	5.8	5.3	5.6	5.1	5.1	5.6
100		4.4	3.9	4.1	4.2	4.7	4.1	3.8	3.9
Significance		Q*	Q*	L**	Q*	L**	L**	L**	Q**

²DAT = days after treatment.

³N concentration averaged over all sampling times.

*, **Linear (L) and quadratic (Q) effects significant at $P \leq 0.05$ and 0.01 .

Fig. 1. Effects of glyphosate rate and stage of development on mineral content of tomato leaves (Expt. 1). Mineral content is expressed as a percentage of leaf dry weight. NS, **Nonsignificant at $P \leq 0.05$, or significant at $P \leq 0.01$, respectively, for linear (L) and quadratic (Q) effects of glyphosate rate.

glyphosate applications, and for 1 and 2 weeks after at-bloom applications. Nitrogen concentration declined linearly from 5.1% with no glyphosate to 4.4% with 100 g·ha⁻¹ glyphosate applied prebloom ($P = 0.0001$, data not shown), when averaged over time of evaluation. Glyphosate applied at bloom did not affect leaf N concentration.

The foliar concentrations of P, K, Ca, and Mg varied with glyphosate rate, stage of development, and time of evaluation. Increased rates of glyphosate from 0 to 100 g·ha⁻¹ applied prebloom decreased leaf P concentration by 23% (Table 4), but had no effect when applied at bloom (data not shown). Unlike the earlier stages of development, the effect of glyphosate applied postbloom on foliar P concentration differed with time of evaluation (Table 4). Response to glyphosate rate was not consistent over the 7- to 28-d evaluation period.

Foliar K concentration decreased linearly with increased rates of glyphosate applied prebloom at all times of evaluation (Table 4), but treatment at bloom had no effect on K concentration (data not shown). Although the initial samples taken 7 d after postbloom application indicated that K concentration increased with glyphosate rate, the response varied with time of evaluation (Table 4).

At 7 and 14 d after prebloom applications, leaf Ca increased with rate of glyphosate; however, at 21 and 28 DAT glyphosate had no significant effect (Table 4). Glyphosate applied at bloom also had no effect on leaf Ca (data not shown). The effect of glyphosate applied postbloom was inconsistent (Table 4).

With prebloom glyphosate applications, Mg concentration increased linearly with rate at all sampled times, with a greater increase occurring at 7 DAT than at later times of evaluation (Table 4). Leaf Mg concentration

increased linearly by 7% as glyphosate, applied at-bloom, increased from 0 to 100 g·ha⁻¹ ($P < 0.01$, data not shown). The leaf Mg response to postbloom glyphosate applications varied with time of evaluation. At 7 DAT, leaf Mg increased linearly with glyphosate rate, but no response was apparent at 28 DAT (Table 4).

Expt. 3. The response of foliar P, K, Ca, and Mg to glyphosate rate differed with stage of development at application and time of leaf sampling ($P \leq 0.0001$). The sensitivity of N, P, and K concentrations to increased rates of glyphosate applied prebloom observed in earlier experiments was not apparent in Expt. 3 (Table 5). The response of leaf P to increased glyphosate rates at all stages of development was cubic. Prebloom applications of 1 to 10 g·ha⁻¹ glyphosate caused P concentrations to decline progressively; however, P concentrations were higher with rates of 60 and 100 g·ha⁻¹ glyphosate than those with 10 g·ha⁻¹ (Table 5). The response to increasing rates of glyphosate applied at bloom was also cubic. Although P concentrations decreased as glyphosate rates increased to 10 g·ha⁻¹, 60 and 100 g·ha⁻¹ glyphosate either increased or had no further effect on P concentration. At 7 DAT, P concentration was unaffected by postbloom glyphosate applications at rates of 10 g·ha⁻¹ or less; however, a marked increase in P concentration occurred with 60 and 100 g·ha⁻¹. In contrast, at 14 DAT, P concentrations increased with 10 g·ha⁻¹ of glyphosate or less applied postbloom, but declined to a low of 0.48% following application of 100 g·ha⁻¹. For leaves sampled 21 and 28 DAT, the lowest P concentrations were observed with 10 g·ha⁻¹ glyphosate.

Seven days after prebloom applications, leaf K first increased with increasing glyphosate rate, then declined with further increases in a quadratic manner (Table 5). At subsequent times of evaluation, glyphosate rates of less than 10 g·ha⁻¹ initially reduced foliar K, which was followed by recovery or an increase in K concentration with 60 or 100 g·ha⁻¹ glyphosate. With glyphosate applications at bloom, K concentration changed significantly at only the first two times of evaluation. Seven days after postbloom glyphosate applications, K concentrations increased linearly with glyphosate rate, but response was inconsistent at subsequent times of evaluation. Similarly for Ca and Mg concentrations, glyphosate application at all three stages of development produced significant responses for some or all times of evaluation; however, response was not consistent over time.

Discussion

The primary mechanism of action of glyphosate is the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, disrupting the biosynthesis of the aromatic amino acids tryptophan, tyrosine, and phenylalanine (Cole, 1985). Symptoms of glyphosate exposure include growth inhibition within days of application and subsequent foliar chlorosis and

necrosis over a 1- to 3-week period (Ahrens, 1994). Therefore, we anticipated that sublethal rates of glyphosate would affect plant vigor and depress plant height, and that rates that produced significant changes in these plant parameters would influence leaf mineral content. The self-limitation of glyphosate translocation has been attributed to a disruption in photosynthesis, leading to reduced translocation of carbon (Geiger and Bestman, 1990; Geiger et al., 1999). Translocation of assimilates out of source leaves and import of assimilates by sink leaves were inhibited. Marschner et al. (1997) reported that cycling and recycling of nutrients such as N, P, K, S, and Mg are closely related to phloem loading and export of photosynthate by source leaves. As a result, we expected that disruptions of photosynthesis and translocation by glyphosate would

affect phloem-mobile elements to a greater extent than the less phloem-mobile Ca.

In the current study, glyphosate application rates of 60 and 100 g·ha⁻¹ consistently reduced plant vigor and height. Similar pronounced changes in leaf mineral content were expected in response to glyphosate, particularly at the earlier stages of development prior to flowering. This was not consistently so and may be due to seasonal differences in sensitivity to glyphosate or other differences in physiological status that caused variable responses to sublethal glyphosate rates. Nutrient absorption occurs throughout the growth and development of tomato plants (Wilcox, 1993), and stunting was observed in this study with 60 and 100 g·ha⁻¹ glyphosate. Thus, the higher concentrations of P in plants treated with 60 and 100 g·ha⁻¹ glyphosate in Expt. 3, than

those treated with 10 g·ha⁻¹, may indicate similar P accumulation but reduced growth at the higher rates. Since the mineral analyses were conducted on the youngest mature leaf, which was sampled at weekly intervals for 4 weeks, the status of sampled leaves as a source or sink at the time of glyphosate application may have contributed to inconsistencies among times of evaluation.

We conclude that tomato leaf mineral content was affected by sublethal rates of glyphosate applied prebloom, at-bloom and postbloom; however, foliar mineral content was a less reliable indicator of sublethal glyphosate exposure than were plant vigor and plant height. Of the minerals assessed, the decrease in leaf N concentration with increasing rates of glyphosate was the most consistent change over the 4-week evaluation period.

Table 4. Effects of glyphosate rate and stage of development on mineral content (% DW) of tomato leaves (Expt. 2).

Rate (g·ha ⁻¹)	DAT ² :	Phosphorus				Potassium				Calcium				Magnesium			
		7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
Prebloom																	
0			0.49 ^y			4.4	4.3	3.8	4.4	1.9	1.9	2.1	2.4	0.64	0.64	0.65	0.74
1			0.50			4.4	4.2	4.3	4.0	2.0	1.8	1.9	2.4	0.68	0.64	0.65	0.75
10			0.48			4.2	4.1	3.8	4.0	1.9	1.8	1.9	2.2	0.64	0.64	0.64	0.74
100			0.38			2.6	3.7	3.1	2.7	2.7	2.1	2.1	2.5	0.93	0.77	0.74	0.81
Significance			L**			L**	L*	L**	L**	L**	L*	NS	NS	L**	L**	L**	L*
Postbloom																	
0		0.45	0.49	0.61	0.39	2.6	3.6	4.3	3.0	1.6	2.3	2.1	2.1	0.56	0.78	0.69	0.67
1		0.41	0.42	0.59	0.32	2.3	3.8	3.9	3.0	1.6	2.3	2.2	1.9	0.53	0.82	0.73	0.66
10		0.52	0.32	0.60	0.57	3.5	2.6	4.4	3.7	2.0	1.8	2.3	2.1	0.63	0.64	0.81	0.65
100		0.49	0.33	0.56	0.53	4.0	2.9	3.9	3.7	1.9	1.6	2.4	2.1	0.68	0.57	0.76	0.67
Significance		Q**	Q**	NS	Q**	Q**	Q**	NS	Q*	Q**	Q**	L*	NS	L**	Q**	Q*	NS

²DAT = days after treatment.

³Phosphorus content with prebloom glyphosate applications averaged over all sampling times.

NS, *, ** Nonsignificant, and significant at $P \leq 0.05$ and 0.01 , respectively, for linear (L) and quadratic (Q) effects.

Table 5. Effects of glyphosate rate and stage of development on mineral content (% DW) of tomato leaves (Expt. 3).

Rate		Phosphorus				Potassium				Calcium				Magnesium			
(g·ha ⁻¹)	DAT ² :	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
Prebloom																	
0		0.54	0.59	0.48	0.43	3.3	3.8	3.8	3.7	2.1	1.8	1.8	2.0	0.69	0.61	0.60	0.66
1		0.54	0.54	0.40	0.41	3.6	4.1	3.4	3.4	2.3	1.8	1.9	2.1	0.69	0.65	0.63	0.66
6		0.53	0.51	0.33	0.29	4.1	3.8	2.5	2.7	2.3	1.9	1.8	2.0	0.71	0.73	0.61	0.63
10		0.39	0.48	0.33	0.27	3.5	3.3	2.7	2.6	2.6	2.1	1.9	2.1	0.94	0.71	0.65	0.67
60		0.60	0.52	0.43	0.33	4.0	3.9	3.8	3.8	1.9	2.0	1.9	2.1	0.63	0.63	0.64	0.66
100		0.56	0.54	0.40	0.35	3.4	4.4	3.9	3.9	1.9	1.9	2.1	2.2	0.66	0.64	0.69	0.70
Significance		C**	C**	C**	C**	Q**	C**	C**	C**	C**	C**	L*	L*	C**	C**	NS	NS
At-bloom																	
0		0.36	0.44	0.38	0.28	4.9	3.7	3.5	3.4	2.2	1.9	2.1	2.7	0.75	0.64	0.68	0.79
1		0.35	0.42	0.37	0.27	4.0	4.0	3.6	3.3	2.2	1.8	2.1	2.6	0.69	0.66	0.72	0.78
6		0.30	0.38	0.29	0.24	3.4	3.3	3.3	3.0	2.5	1.8	2.1	2.6	0.72	0.64	0.74	0.79
10		0.23	0.36	0.30	0.23	2.8	3.3	3.4	3.0	2.3	1.8	2.1	2.6	0.68	0.65	0.73	0.82
60		0.44	0.39	0.29	0.25	3.9	3.8	3.7	2.9	2.0	2.0	2.8	3.4	0.63	0.65	0.78	0.83
100		0.44	0.38	0.27	0.25	3.7	3.7	3.7	2.9	1.9	2.1	2.6	3.4	0.65	0.69	0.78	0.87
Significance		C**	C**	C**	C**	C**	C**	NS	NS	L**	L**	C*	C**	L**	NS	L**	L**
Postbloom																	
0		0.23	0.58	0.62	0.60	2.9	3.7	4.6	4.1	3.3	2.0	1.9	1.8	0.86	0.70	0.70	0.65
1		0.22	0.60	0.61	0.53	3.0	3.9	4.5	3.3	3.2	2.0	1.9	1.8	0.87	0.81	0.69	0.66
6		0.23	0.65	0.57	0.33	2.9	5.2	4.3	3.7	3.4	1.9	1.8	2.5	0.95	0.66	0.66	0.78
10		0.22	0.63	0.40	0.30	2.8	5.0	2.7	3.0	3.1	1.8	2.1	2.5	0.89	0.63	0.73	0.72
60		0.61	0.58	0.60	0.33	3.7	4.5	4.7	4.5	2.1	1.8	1.8	2.8	0.70	0.68	0.65	0.82
100		0.67	0.48	0.58	0.35	4.5	3.2	4.6	3.7	2.1	1.9	1.7	3.0	0.71	0.78	0.64	0.86
Significance		C**	C**	C**	C**	L**	C**	C**	C*	Q**	NS	NS	C**	C*	C*	NS	L**

²DAT = Days after treatment.

NS, *, ** Nonsignificant and significant at $P \leq 0.05$ and 0.01 , respectively, for linear (L), quadratic (Q), or cubic (C) effects.

Consistency of response over time is important for diagnostic purposes since the time of exposure is often unknown and physical symptoms of glyphosate injury on foliage often develop slowly. A more direct measurement of the presence of glyphosate is determination of shikimate content (Singh and Shaner, 1998) and this may serve as a more reliable tool than leaf mineral analysis. Although significant changes in foliar N, P, K, Ca, and Mg were observed in response to sublethal rates of glyphosate, mineral deficiencies (Maynard and Hochmuth, 1997) did not occur at rates up to 100 g·ha⁻¹.

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