Turfgrass species response exposed to increasing rates of glyphosate application

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ARTICLE INFO

Article history:
Received 20 May 2008
Received in revised form 12 May 2009
Accepted 14 May 2009

Keywords:
5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS)
Glyphosate
Mineral nutrients
Shikimate
Turfgrass

ABSTRACT

To investigate the response of nine turfgrass species exposed to increasing rates of glyphosate application, the dry matter production, visual leaf injury symptoms (e.g., chlorosis and necrosis) and the concentrations of shikimate and mineral nutrients were determined in shoots. The rates of foliar glyphosate application were 0%, 5% (1.58 mM), and 20% (6.32 mM) of the recommended application rate for weed control. In general, there was a negative and weak correlation between the intensity of visual injury and relative decreases in shoot dry matter production caused by glyphosate application. The decreases in shoot dry matter production and the severity of leaf damage pronounced by increasing glyphosate rate showed a substantial variation among the turfgrass species. Of the turfgrass species tested, *Festuca arundinacea* 'Falcon' and *Buchloe dactyloides* 'Bowie' were selected as the most tolerant and sensitive species to applied sublethal rates of glyphosate as judged from visual injury ratings, respectively. At the highest glyphosate rate, shoot dry weight was decreased by 4-fold in Bowie and only 1.6-fold in Falcon. When glyphosate was not applied, shoot shikimate concentration of all species was very low and below 2.8 μmol g^{-1} FW (fresh weight). Glyphosate applications resulted in increases in shoot shikimate concentration with substantial variations among species. At 6.32 mM glyphosate treatment, shikimate concentration ranged between 156.1 μmol g^{-1} (*F. rubra*, Ambrose) and 16.5 μmol g^{-1} FW (*F. rubra*, Cindy Lou). However, the highly sensitive and the tolerant genotypes were not different in shoot shikimate concentrations. Even, in the case of some genotypes, high glyphosate tolerance is accompanied by higher shoot concentrations of shikimate. Depending on the turfgrass species and mineral nutrients tested, increasing glyphosate application either did not affect or reduced mineral nutrient concentrations. In the case of decreases in shoot concentration of mineral nutrients, the decreases in Ca, Mg, Mn and Fe were most distinct. The results obtained indicate existence of a large genetic variation in tolerance to glyphosate toxicity among the turfgrass species. This differential variation in tolerance to glyphosate could not be explained by the changes in shoot concentrations of shikimate and mineral nutrients.

1. Introduction

Glyphosate is a broad spectrum, systemic, non-selective, post-emergence herbicide which was commercialized in 1974, for the control of annual and perennial weeds. Weed growth is affected by blocking aromatic amino acid production, leading to the arrest of protein production and the prevention of secondary compound formation. Glyphosate competes with phosphoenolpyruvate (PEP) and inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a nuclear encoded chloroplast-localized enzyme in the shikimic acid pathway of higher plants, bacteria, algae, and fungi (Steinrücken and Amrhein, 1980; Kishore and Shah, 1988). Moreover, the herbicide causes a rapid increase in shikimic acid and to a lesser extent, shikimate derived benzoic acids. This increase in shikimic acid has been related to a decline in carbon fixation intermediates (Duke et al., 2003). Grass management and production is one of the fastest growing areas of agriculture. Creeping bentgrass (*Agrostis* spp.), Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea*) are among the cool-season grasses which are commonly used in recreational areas including golf courses, tree areas and fairways. On the other hand, warm-season grasses such as buffalograss (*Buchloe dactyloides*) and zoysiagrass (*Zoysia* spp.) with their lower evapotranspiration rates and better drought resistance characteristics (Huang and Fry, 1999) are alternatives to cool-season grasses which are more vulnerable to a wide range of pests. Various weedy grasses, including annual bluegrass (*Poa annua* L.), are serious management problems in bentgrass fairways and putting greens (Turgeon, 2002). In order to simplify and improve the control of grass and broadleaf species that can invade recreational areas, genetically engineered glyphosate resistant creeping bentgrass cultivars have been developed by heterologous expression of a gene from the CP4 strain of *Agrobacterium* sp. encoding for a glyphosate resistant form of EPSPS (Reichman et al., 2006). More-

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Table 1
Visual injury scoring and dry matter production of turfgrass species nine days after glyphosate treatments of 5% and 20% of the recommended rate for weed control corresponding to 1.58 and 6.32 mM glyphosate, respectively.

Turfgrass species	Visual injury score		Dry matter Production (mg plant^{-1})		
	5% Glyp.	20% Glyp.	control	5% Glyp.	20% Glyp.
A. canina, Greenwich	10	60	3.4	1.9	1.0
A. stolonifera, PennA4	20	80	4.2	1.7	1.4
A. tenuis, Tiger II	0	75	2.1	1.4	1.3
B. dactyloides, Bowie	60	100	73.3	44.0	18.0
F. arundinacea, Falcon	10	40	38.6	36.4	23.2
F. rubra subsp. *falax*, Ambrose	0	100	147.5	71.0	56.3
F. rubra subsp. *littoralis*, Cindy Lou	30	60	14.9	13.7	8.9
L. perenne, Charismatic	20	90	24.0	19.2	14.1
P. pratensis, Midnight	60	100	4.9	4.3	4.0
Mean	23	78	34.8	21.5	14.2
$LSD_{0.05}$(S, G, S × G)	(4.9, 2.3, 6.9)				

The visual herbicidal injury symptoms (i.e., chlorosis and necrosis) on plant shoots were scored on a 0–100 scale as 0 indicating no injury symptom and 100 indicating a total pigment loss and inhibition of growth. (S, species; G, glyphosate; S × G, species × glyphosate interaction).

over, glyphosate is also used in converting putting greens from one turfgrass species or cultivar to another in putting greens (Zuk and Fry, 2005). For the management of turfgrass species, there is an increased reliance on the herbicide glyphosate for control, due to widespread evolution of resistance to other herbicides with different modes of action (Baylis, 2000). While developing future management strategies to both slow evolution of resistance and to control existing populations, a better understanding of the herbicide action on turfgrass species would be essential. Several reports are available showing existence of a large genetic variation within and among grass species for tolerance to glyphosate toxicity (Lym et al., 1991; Tardif and Leroux, 1991, 1993; Webster et al., 2004). However, the morphological or physiological reasons of such wide genetic variation in tolerance to glyphosate are not well understood. One possible explanation could be related to differences in leaf penetration and translocation of glyphosate into growing parts of plants (Tardif and Leroux, 1991, 1993; Nalewaja and Matysiak, 1991). Mineral nutrient concentrations of plants could also affect the toxic action of glyphosate by interfering with its physiological availability. High concentrations of some cationic nutrients in spray solutions significantly reduce phytotoxic effects of glyphosate through formation of poorly soluble glyphosate complexes (Schonherr and Schreiber, 2004; Thelen et al., 1995).

This study was conducted to investigate the effect of increasing rates of foliar glyphosate applications on shoot dry matter production and shoot concentrations of mineral nutrients and shikimate in selected turfgrass species. The visual injury symptoms caused by glyphosate application were also evaluated.

2. Materials and methods

2.1. Plant materials and growing conditions

Nine turfgrass species (Table 1) were planted in 15-cm diameter plastic pots containing a mixture of 35% peat, 32% vermiculite, 9% soil, and 24% sand (v/v) and grown in a greenhouse in a randomized block design with three replications. For basal fertilization the growth media was treated with 200 mg kg^{-1} N, 100 mg kg^{-1} P, 50 mg kg^{-1} K and 20 mg kg^{-1} S. Greenhouse conditions were maintained at approximate day/night temperatures of 25/20 °C (±3). Glyphosate, formulated as Roundup Ultra [active ingredient (a.i.): 480 g L^{-1} *N*-[phosphonomethyl] glycine, Monsanto Co.] was supplied from Monsanto Ltd., Adana, Turkey, and was used in the experiments.

For evaluation of the physiological responses of turfgrass species upon exposure to glyphosate, selected turfgrass species were treated with two different rates of glyphosate. The rates were determined according to the recommended application rate provided on the product label for narrow and broad leaf annual weeds (i.e. 1.44 kg ha^{-1} a.e. glyphosate to be applied with 200 L of water per ha: 31.55 mM glyphosate as active ingredient). The applied rates were 5% (1.58 mM), and 20% (6.32 mM) of the recommended rate for the control of annual weeds. In all experiments, plants at the three-leaf growth stage were sprayed with freshly prepared glyphosate solution until all leaves were fully wet (about 10 ml) but without run-off. Control plants were sprayed with distilled water.

Following 9 days after treatment (9 DAT) plants were visually scored to estimate the herbicidal injury due to glyphosate applications on a scale of 0–100 with 0 indicating no visual injury and 100 indicating a total pigment loss.

2.2. Shoot dry matter production and mineral nutrient concentration

For determination of shoot dry matter and mineral nutrients, whole shoots were harvested randomly from each pot at nine days after foliar glyphosate treatments (9 DAT), and dried at 65 °C until a constant weight. After grinding, 200 mg of sample was digested using a closed vessel digestion system (MarsExpress; CEM Corp., Matthews, NC, USA) in 65% (w/v) HNO_3. Following digestion, concentration of mineral nutrients was measured by inductively coupled plasma-optical emission spectrophotometry (ICP-OES) (Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia).

2.3. Determination of shikimate concentration

The shoot shikimate concentration was determined using the method of Cromartie and Polge (2000). Shoot samples (0.3–0.5 g FW) were randomly harvested at 5 and 9 days after foliar glyphosate treatments. The harvested plant materials were frozen in liquid nitrogen and stored at −80 °C. Extracts were stored at −80 °C for a maximum of one week before analysis. This was done as outlined by Shaner et al. (2005).

2.4. Statistical analysis

In all experiments, each treatment consisted of three independent replications and the experiments were repeated twice using randomized complete block design. Analysis of variance procedure in JMP statistical package (version 5.0.1a, SAS Institute Inc., Cary, NC, 1989–2002) was used to test for treatment effects. Means were separated by Tukey's least significant difference test when a significant ($P \leq 0.05$) difference occurred.

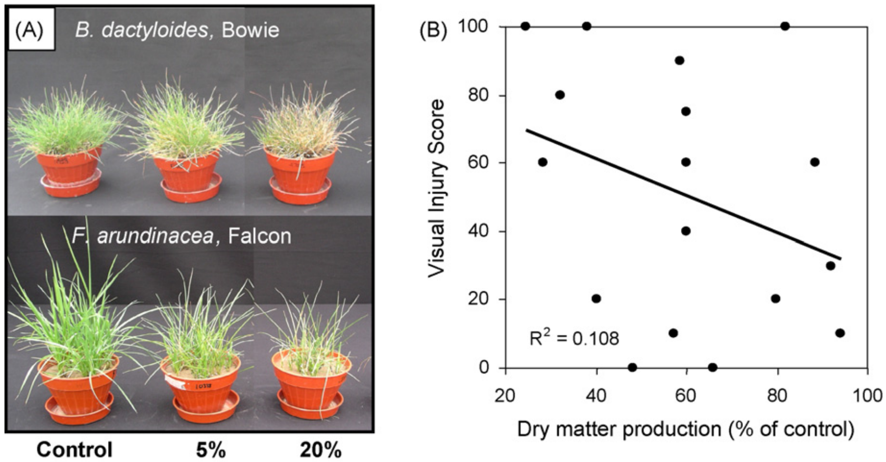

Fig. 1. (A) Growth of the most susceptible (*B. dactyloides*, Bowie) and most tolerant (*F. arundinacea*, Falcon) turfgrass species selected by visual injury scoring at nine days after glyphosate treatments (5% and 20% of the recommended rate for weed control) and (B) relationship between visual injury rates and dry matter production of turfgrass species.

3. Results

3.1. Visual injury scoring and shoot dry matter production

The visual injury of plants (Table 1) was scored to determine the magnitude of the leaf damage by glyphosate. As expected, the injury scores were significantly increased with increasing glyphosate rates. Visual injury symptoms of chlorosis and necrosis were evident in all turfgrass species as early as 2 DAT. Among the turfgrass species tested, Falcon (*F. arundinacea*) was the most tolerant as judged by the visual injury scoring at 20% glyphosate treatment (Table 1). At this glyphosate rate, more than half of the Falcon plants could survive (Fig. 1A). Similar to Falcon, *F. rubra* subsp. *littoralis*, Cindy Lou and *A. canina*, Greenwich also expressed lower injury symptoms compared to other turfgrass species, particularly at the higher glyphosate rates (i.e. 20%). Among all the genotypes

Table 2
Effect of increasing glyphosate rates (5% and 20% of the recommended rate for weed control) on mineral nutrient concentration of turfgrass species. Least significant difference ($LSD_{0.05}$) at $\alpha = 0.05$ is calculated for mean values across species.

Species/cultivars	Glyphosate	Ca (%)	K (%)	Mg (%)	P (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
A. canina, Greenwich	Control	0.43	1.74	0.18	0.26	5.49	44.7	134.3	33.9
	5%	0.50	1.50	0.20	0.27	5.32	44.2	161.6	38.2
	20%	0.56	1.91	0.21	0.33	6.14	38.5	181.4	39.2
A. stolonifera, PennA4	Control	0.52	2.00	0.17	0.32	7.65	42.7	82.5	35.4
	5%	0.49	1.54	0.15	0.32	6.04	39.4	81.0	30.9
	20%	0.58	1.74	1.7	0.37	7.79	46.1	109.4	35.5
A. tenuis, Tiger II	Control	0.67	2.28	0.23	0.27	8.87	58.6	28.0	60.9
	5%	0.54	1.76	0.21	0.28	8.36	55.0	111.1	61.5
	20%	0.48	2.28	0.18	0.37	9.86	62.0	120.2	60.1
B. dactyloides, Bowie	Control	0.61	1.11	0.20	0.23	8.06	60.9	75.5	61.7
	5%	0.50	1.24	0.16	0.27	7.19	48.4	59.1	54.2
	20%	0.41	1.03	0.14	0.35	4.91	53.8	61.1	56.4
F. arundinacea, Falcon	Control	0.62	2.47	0.36	0.45	12.89	91.8	96.3	100.0
	5%	0.33	1.22	0.19	0.22	5.36	46.0	37.5	39.9
	20%	0.29	1.15	0.16	0.291	5.18	41.4	28.9	41.8
F. rubra subsp. *falax*, Ambrose	Control	0.91	2.14	0.25	0.43	9.92	64.5	103.2	68.3
	5%	0.79	2.00	0.21	0.28	9.05	48.2	101.1	47.8
	20%	0.98	1.97	0.25	0.30	9.76	68.5	108.8	59.8
F. rubra subsp. *littoralis*, Cindy Lou	Control	0.60	2.34	0.22	0.38	8.63	69.1	83.7	67.6
	5%	0.81	1.31	0.20	0.22	5.96	43.2	49.7	42.8
	20%	0.76	2.07	0.22	0.41	8.89	70.1	59.8	61.6
L. perenne, Charismatic	Control	0.55	2.03	0.3	0.34	7.72	108.3	53.0	47.8
	5%	0.33	1.41	0.17	0.24	4.63	54.5	32.0	31.4
	20%	0.38	1.35	0.16	0.21	5.84	64.9	33.9	41.7
P. pratensis, Midnight	Control	0.59	2.27	0.26	0.49	14.14	94.6	70.8	47.7
	5%	0.76	1.82	0.27	0.29	8.45	63.3	50.6	46.3
	20%	0.77	2.22	0.29	0.38	10.50	85.2	57.9	48.8
Mean	Control	0.61	2.04	0.24	0.35	9.26	70.6	91.9	58.2
	5%	0.56	1.53	0.20	0.27	6.71	49.1	76.0	43.7
	20%	0.58	1.75	0.20	0.32	7.65	58.9	84.6	49.4
$LSD_{(0.05)}$		0.05	0.06	0.01	0.01	0.4	3.4	4.8	2.7

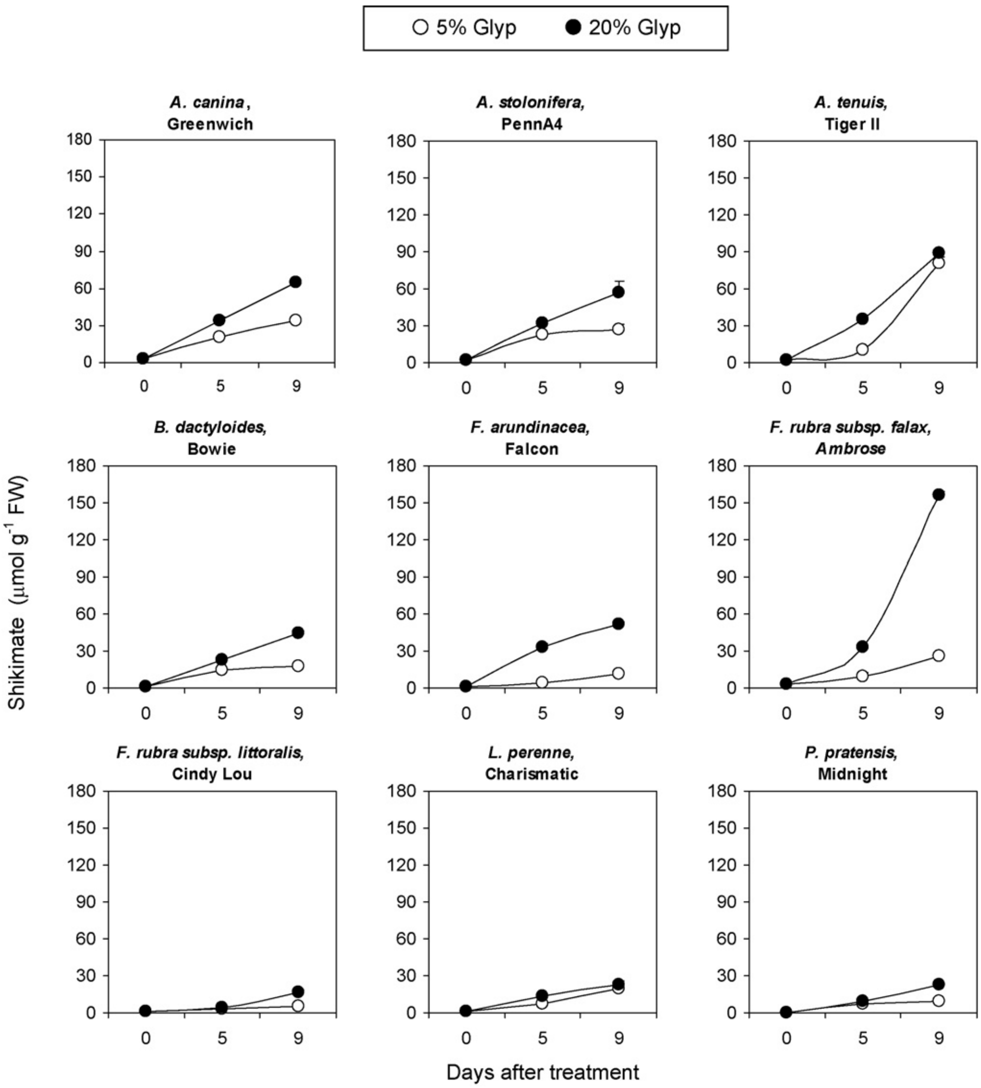

Fig. 2. Effect of increasing glyphosate rates (5% and 20% of the recommended rate for weed control) on leaf shikimate concentration of turfgrass species. Bars represent the standard deviation of each mean. Where bars are absent, they are obscured by the data symbol.

tested, *B. dactyloides* cv. Bowie was the most sensitive and received the highest visual injury score in all the glyphosate rates tested (Table 1, Fig. 1A). Interestingly, the genotype Tiger II expressed a high sensitivity to glyphosate application regarding the severity of leaf damage but showed little decrease in shoot dry matter production by glyphosate application (Table 1). This cultivar also showed either no or very slight symptoms at 5% glyphosate applications, whereas it was ranked as sensitive due to the rapidly enhanced injury symptoms at 20% glyphosate treatment. A similar rapid symptom evolution was also evident in *F. rubra* subsp. *falax*, Ambrose which had symptom scores of 0 at 5% compared to 100 at 20% glyphosate treatments.

There was a poor ($P < 0.19$) and negative relationship between visual injury scores and relative decreases in shoot dry matter production (Fig. 1B). Visual injury scoring alone was not a suitable selection criterion for tolerance to glyphosate toxicity in the turfgrass species tested.

There were large differences in shoot dry matter yield among the genotypes under all glyphosate treatment (Table 1). Ambrose and Tiger II were the genotypes producing the highest and lowest shoot dry weight under given conditions without glyphosate application, respectively. The genotypes Bowie, PennA4, and Greenwich were the most severely affected genotypes at all glyphosate rates compared to their control plants. On the contrary, Midnight, Charismatic, Cindy Lou, Tiger and Falcon genotypes were lesser affected from glyphosate application when compared to their control (untreated plants).

3.2. Shoot concentration of mineral nutrients

Depending on the grass species tested, increasing glyphosate applications differentially affected the shoot concentrations of mineral nutrients (Table 2). The most distinct effect of glyphosate was its decreasing effects on mineral nutrient concentrations in some genotypes. In the case of the genotypes Falcon, Bowie and Charismatic, glyphosate applications reduced shoot concentrations of mineral nutrients, especially Ca, Mg, Fe and Mn (Table 2). Generally, the shoot concentrations of K, P, Cu and Zn were less affected from increasing glyphosate applications.

3.3. Shikimate concentrations

When glyphosate was not applied, the shikimate concentrations were between 0.6 and 2.8 $\mu mol\,g^{-1}$ fresh weight (FW) for all turfgrass species tested (Fig. 2). The *Agrostis* species (e.g., the genotypes Greenwich, PennA4 and Tiger II) had higher amounts of shikimate concentration (1.7–2.6 μmol shikimate g^{-1} FW) without any glyphosate application among the turfgrass species. Plants responded to increasing glyphosate applications with marked increases in shoot shikimate concentrations. The highest concentration of shikimate was 156.1 μmol g^{-1} FW detected in Ambrose at 9 DAT with 20% glyphosate application (Fig. 2). In the Agrostis species, shikimate concentration increased approximately 10-fold at 20% glyphosate application compared to their basal levels at 5 DAT. In general, shikimate concentration continued to increase at 5 and 20% glyphosate applications in all species tested until 9 DAT, but the level of this increase was highly different among species and genotypes.

Festuca species had generally lower amounts of basal shikimate concentration (0.6–0.9 μmol shikimate g^{-1} FW) among the studied turfgrass species with no glyphosate treatment, with the exception of *F. rubra* sp. *falax* 'Ambrose' (2.8 μmol shikimate g^{-1} FW). Five days after the glyphosate treatment, the *Festuca* species began to show significant differences in shikimate concentration for two different rates of glyphosate applied. Following 5 DAT, the shikimate concentration in *F. rubra* sp. *falax* 'Ambrose' had increased approximately 43 and 48 times than that of basal level, while in *F. rubra littoralis* 'Cindy Lou' and *F. arundinacea* 'Falcon' this increase was 24–29-fold at the 20% of the labeled glyphosate rate, respectively (Fig. 2). At 20% glyphosate application, *F. rubra littoralis* 'Cindy Lou' plants tolerated the dose well, with shikimate concentration not increasing significantly after 5 DAT. However, at 9 DAT, the concentration of shikimate increased by ~4-fold compared to that of found at 5 DAT.

L. perenne 'Charismatic' and *P. pratensis* 'Midnight' species had low levels of shikimate concentrations in untreated plants (0.5–0.6 μmol shikimate^{-1} g FW) (Fig. 2). An approximate 8-fold increase in shikimate concentration was observed in *L. perenne* 'Charismatic' species for the 5% glyphosate application at 9 DAT, while only an increase of ~2-fold was determined for *P. pratensis* 'Midnight'.

B. dactyloides 'Bowie' species control plants had moderate amounts of shikimate (0.6 ± 0.05 [mean ± SD] μmol shikimate^{-1} g FW) among the studied turfgrass species. However, at 5 DAT ~10-fold increase in shikimate concentration was observed for the 5% of the labeled rate application of glyphosate. *B. dactyloides* 'Bowie' showed significant differences in shikimate concentration for different rates of glyphosate applied at 5 DAT. An approximate 4-fold increase in shikimate concentration was observed at 9 DAT with 5% glyphosate application.

4. Discussion

Glyphosate is a commercial pesticide used in control of weed species which exerts its action on plants through inhibition of EPSPS. Although glyphosate was considered as a low risk herbicide to evolve resistant weeds (Pratley et al., 1999), many recently evolved glyphosate resistant species including grasses have been reported all over the world (Powles et al., 1998; Baerson et al., 2002; Perez and Kogan, 2003; Perez-Jones et al., 2005; Heap, 2007). In order to develop strategies for the future management practices of grass species to slow down the evolution of resistance and control existing populations, action of the herbicide on grass species should be assessed in detail. In this research, the variation in response of selected turfgrass species to glyphosate was studied at the physiological level.

F. arundinacea 'Falcon' and *B. dactyloides* 'Bowie' were selected as the most tolerant and sensitive species to applied sublethal rates of glyphosate as determined with visual injury ratings, respectively. *B. dactyloides* 'Bowie' has pubescent (soft erect hairs) leaves which might have increased herbicide penetration. The dry matter production in leaves of turfgrass species decreased with increasing rates of glyphosate applied. There was a negative correlation between visual injury scores and dry matter production indicating that the outbreak of glyphosate injury symptoms such as chlorosis and necrosis is associated with inhibition of biomass production under glyphosate pressure. However, this relationship was fairly weak for the turfgrass species tested. Published data in literature show that there are numerous controversial results in terms of correlation between leaf injury and changes in growth or yield upon herbicide application (Obrigawitch et al., 1998). In most cases changes in yield or growth in response to application of sulfonylurea herbicides or other post-emergence herbicides were not related to severity of leaf symptoms as shown in various plant species (Lemerle et al., 1985; Leys et al., 1987; Walsh et al., 1993; Fletcher et al., 1993).

Glyphosate is known to have high binding affinity to metals (Undabeytia et al., 2002; Barja et al., 2001) and can affect uptake of metal cations, although whether the effect is direct or indirect in the field is not clear. Frequent applications of glyphosate to soybean (Glycine max) were found to induce Fe, Zn, and Mn deficiencies based on reports from field observations (Franzen et al., 2003; Jolley et al., 2004). In a short term radiolabeled uptake study (Eker et al., 2006), it was reported that root-to-shoot translocation of micronutrients were severely inhibited in the order of Mn > Fe > Zn in sunflower plants. Recently, root ferric reductase activity of Fe-deficient sunflower plants was shown to be inhibited by drift rates of glyphosates providing possible evidence for glyphosate-induced chlorosis observed in field grown Strategy-I type Fe-utilizing plant species (Ozturk et al., 2008). There are a number of investigations on the antagonistic effects of glyphosate on cationic micronutrients (Franzen et al., 2003; Jolley et al., 2004; Eker et al., 2006). Glyphosate is also inactivated by cationic nutrients such as Ca and Mg in spray solutions due to formation of insoluble glyphosate complexes with Ca and Mg (Thelen et al., 1995; Bailey et al., 2002). When applied to foliage, glyphosate is rapidly transported to roots and even released from roots into the rhizosphere (Kremer et al., 2005). It is therefore possible that glyphosate may interfere with both uptake and root-to-shoot transport of cationic nutrients by formation of insoluble glyphosate complexes, as shown for Fe and Mn (Eker et al., 2006). In the present study, it was found that glyphosate application at 5% level generally decreased all cationic macro- and micronutrients measured in the leaf tissue except Greenwich and Cindy Lou. However, this reduction was restored to some extent at the 20% rate of glyphosate applications, possibly due to the concentration effect associated with severe shoot damage and reduction in shoot biomass production under the highest glyphosate rate (Table 1). Interestingly, the genotypes Bowie and Falcon with highest and lowest susceptibility to glyphosate, respectively, showed most distinct decreases in shoot concentrations of mineral nutrients by glyphosate, especially with Ca, Mg, Mn and Fe (Table 2). This result indicates that impairment in mineral nutritional status of plants is not involved in differential expression of glyphosate tolerance among the turfgrass species. In velvetleaf, elevated glyphosate tolerance was ascribed to high concentrations of divalent cations (e.g. Ca, Mg and Mn) in the leaf surface (Nalewaja et al., 1996). It is suggested that by complexing glyphosate, divalent cations interfere with absorption and translocation of glyphosate in plants. In the present study, Ambrose and Charismatic genotypes had the highest and lowest concentrations of Ca among all turfgrass species, respectively (Table 2), but they were similarly sensitive to glyphosate (Table 1).

Glyphosate specifically inhibits the EPSPS enzyme from shikimic acid pathway, resulting in an unregulated flow of carbon to be diverted into intermediates upstream of the blocked EPSPS enzyme, mainly shikimic acid (Jensen, 1985). The elevated levels of shikimate have been widely considered as a reliable indicator for the magnitude of the glyphosate toxicity (Harring et al., 1998; Shaner et al., 2005). In the present study, there was, however, no relationship between accumulation of shikimate (Fig. 2) and visual injury caused by phytotoxicity of glyphosate (Table 1). The most tolerant (Falcon) and sensitive (Bowie) turfgrass genotypes were very similar in accumulation of shikimate (Fig. 2). Similarly, at 5% of the labeled glyphosate rate, Midnight was highly susceptible (injury score: 60) while Tiger 2 was not affected (injury score: 0); but, the shikimate concentration of Tiger II was substantially higher than the shikimate concentration found in Midnight (Table 1; Fig. 2) These results indicate that there are additional physiological process which are also affected by glyphosate applications, and clearly, this issue needs further investigation. For instance, aminomethylphosphonic acid (AMPA) was suggested to be the main compound responsible for glyphosate-induced injury in plants (Reddy et al., 2004) that might be measured in the future studies.

The results of this study clearly indicate that the differential expression of glyphosate tolerance among the turfgrass species is not related to differences in shoot concentrations of mineral nutrients and shikimate. It appears that glyphosate toxicity in turfgrass species cannot be solely ascribed to inhibition of EPSP synthase enzyme. A similar observation was also made in horseweed by Mueller et al. (2003). The levels of shikimate concentration in plant tissues cannot be considered as a reliable physiological parameter for the extent of the glyphosate phytotoxicity. In most cases, genotypic variation in glyphosate tolerance is studied by using two or three genotypes or species. The present study points out that the tolerance mechanisms against glyphosate toxicity may vary significantly from one species to another.

Acknowledgements

This research was supported in part by the European Commission's Marie Curie International Reintegration Grant (FP6). We owe our special thanks to Drs. Robert (Bob) Shearman and Stacy Bonos for providing the plant materials.

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