Aminomethylphosphonic Acid Accumulation in Plant Species Treated with Glyphosate

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INTRODUCTION

Glyphosate degrades relatively rapidly in soils by microbial processes (6). The most frequently detected degradation product in soil and water is aminomethylphosphonic acid (AMPA). Little is known about the enzyme(s) involved in degradation of glyphosate to AMPA in plants. Although glyphosate is minimally metabolized by plants (4), AMPA is found as a major metabolite in seeds of canola (2), field pea, barley, flax (3), and glyphosate-resistant (GR) soybean treated with glyphosate (1, 5).

Detection of AMPA in glyphosate-treated plants suggests that a plant glyphosate oxidoreductase (GOX) or similar type of enzyme catalyzes this conversion. AMPA is phytotoxic to soybean, although less active than glyphosate; its mode of action is apparently different from glyphosate, because GR and non-GR soybeans are equally sensitive to AMPA (7). GOX has been characterized in several genera of bacteria; however, no plant-derived GOX has been described in the literature. Some leguminous species are more resistant to glyphosate than others. Low levels of resistance to glyphosate in certain leguminous species may be due to differences in GOX activity. In this research, we investigated if other leguminous species also produce AMPA from glyphosate.

OBJECTIVES

To determine differences in glyphosate sensitivity by measuring I_{50} rates (rate required to cause 50% reduction in plant growth) and to quantify glyphosate, AMPA, and shikimate concentrations in GR soybean, GR corn, Italian ryegrass, and several broadleaf weeds treated with glyphosate at their respective I_{50} rate.

MATERIALS AND METHODS

General Experimental Conditions: Greenhouse experiments were conducted using eleven plant species with varying levels of sensitivity to glyphosate (Table 1). Plants were grown in 10-cm diameter plastic pots. Greenhouse was maintained at 28/22º C day/night temperature with natural light supplemented by sodium vapor lamps to provide 13-h photoperiod. Plants at 1- to 6-leaf stage (except horseweed, 25-leaf stage) were sprayed with glyphosate using an indoor spray chamber.

Glyphosate I_{50}: Glyphosate acid (technical grade) at 0, 0.03, 0.06, 0.12, 0.25, 0.50, 1.00, 2.00, and 4.00 kg/ha with Tween 20 (0.5%, v/v) was applied to plants. At 14 days after treatment (DAT), shoot fresh weights were recorded. Treatments were arranged in a randomized complete block design with 5 or 6 replications. Data were fitted to a sigmoidal logistic models to relate percent shoot fresh weight reduction (*y*) to herbicide rate (*x*) as shown in Figures 1 and 2. Glyphosate at 170 g/ha for horseweed and 220 g/ha for Italian ryegrass were used as I_{50} rate.

Glyphosate, AMPA, and Shikimate Accumulation in Glyphosate Treated Plants: Glyphosate, AMPA, and shikimate levels were quantified following treatment with I_{50} rates of glyphosate. At 7 DAT, plants were clipped at the soil surface, washed with water, and blotted dry with paper towels. Treated plant samples consisted of both stem and leaves. All plant samples were air-dried, ground, and analyzed for glyphosate, shikimate, and AMPA. There were 5 to 7 pots per treatment and treatment was replicated five times. Glyphosate, AMPA, and shikimate analysis was performed as described before (7). Glyphosate and AMPA were derivitized and analyzed by GC-MS. Shikimic acid was analyzed by HPLC. Glyphosate, AMPA, and shikimic acid were quantitated from a calibration curve of standards on duplicate samples.

RESULTS

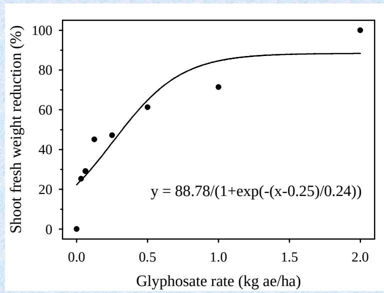

Figure 1. Response of non-glyphosate-resistant soybean to glyphosate at 2 weeks after treatment.

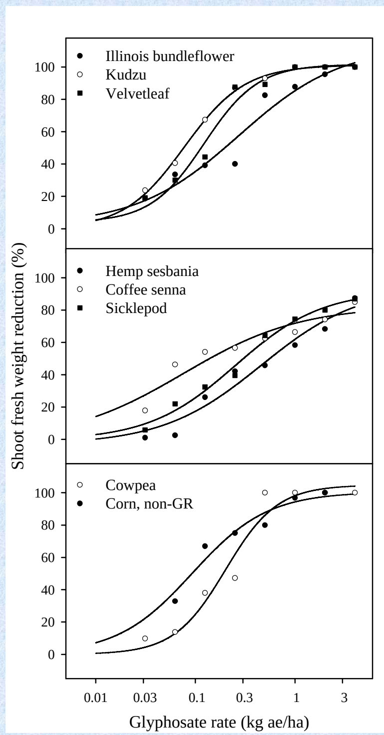

Figure 2. Response of eight plant species to glyphosate at 2 weeks after treatment.

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Table 1. Effect of glyphosate at I_{50} rate on glyphosate, aminomethylphosphonic acid (AMPA), and shikimate concentration at 7 days after treatment in several plant species.[a]

				Shikimate	
Plant species[b]	Glyphosate[c] I_{50}	Glyphosate	AMPA	Glyphosate treated	Non treated
	g ae/ha	ng/g tissue	ng/g tissue	µg/g tissue	µg/g tissue
Soybean, GR	250	5,826	119	53	65
Soybean, non-GR	250	25,036	668	15,251	27
Cowpea	201	26,763	4,765	16,530	64
Sicklepod	252	6,414	1,834	1,092	348
Coffee senna	75	5,906	287	686	277
Hemp sesbania	456	38,650	Nd[d]	6,828	266
Illinois bundleflower	272	3,274	1,513	668	21
Kudzu	77	5,561	297	8,915	188
Velvetleaf	122	678	Nd	159	2
Horseweed	170	26,326	314	9,101	251
Corn, GR	93	308	Nd	245	241
Corn, non-GR	93	851	Nd	149	161
Italian ryegrass	220	7,432	Nd	6,701	904

[a] I_{50}, glyphosate rate required to cause a 50% reduction in plant growth.

[b] GR, glyphosate-resistant; non-GR, non glyphosate-resistant.

[c] Glyphosate at 250 and 93 g/ha were used for GR soybean and corn, respectively, to enable comparison between GR and non-GR types. Tween 20 at 0.5% (v/v) was added to all treatment solutions.

[d] Nd, peak not detected or peak below limit of quantitation.

CONCLUSIONS

1. Coffee senna was the most sensitive (I_{50}, 75 g/ha) and hemp sesbania was the most resistant (I_{50}, 456 g/ha) to glyphosate.
2. Hemp sesbania was six-fold and Illinois bundleflower was four-fold more resistant to glyphosate than coffee senna.
3. Glyphosate was present in all plant species and its concentration ranged from 0.308 to 38.7 µg/g of tissue.
4. AMPA concentration ranged from 0.119 to 4.77 µg/g of tissue. AMPA was detected in six of the seven leguminous species studied.
5. Shikimate was present in all plant species treated with glyphosate and levels ranged from 0.053 to 16.5 mg/g of tissue.
6. Non-glyphosate-resistant (non-GR) soybean accumulated much higher shikimate than glyphosate-resistant (GR) soybean.
7. These results suggest that some leguminous species are more resistant to glyphosate than others, and a plant glyphosate oxidoreductase (GOX) may be responsible for breakdown of glyphosate to AMPA.

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