

## Potato Response to Simulated Glyphosate Drift

Joel Felix, Rick Boydston, and Ian C. Burke\*

Field studies were conducted in 2008 in Ontario, OR and Paterson, WA to determine the effect of simulated glyphosate drift on 'Ranger Russet' potato, including visual injury, shikimic acid accumulation, and tuber yield. Glyphosate was applied at 8.5, 54, 107, 215, and 423 g ae ha<sup>-1</sup>; which corresponds to 0.01, 0.064, 0.126, 0.254, and 0.5 of the lowest recommended (846 g ha<sup>-1</sup>) single application dose for glyphosate-resistant corn and sugar beet. Glyphosate was applied when potato plants were at 10-cm height, stolon hooking, tuber initiation, or bulking stage. The greatest visual foliar injury was observed when glyphosate was applied at a dose of 54 g ha<sup>-1</sup> or greater and potato plants were at the hooking stage. The lowest foliar injury was observed when glyphosate was applied to potato plants at the bulking stage. The I<sub>50</sub> glyphosate dose at 42 d after treatment (DAT) was estimated to be 167 g ha<sup>-1</sup> for potatoes sprayed at the hooking stage. The corresponding glyphosate dose to result in 50% injury for potatoes sprayed at tuber initiation, 10-cm height, and bulking stages were 129%, 338%, and 438%, respectively, greater than hooking stage. The U.S. No.1 potato yield was inversely related to vine injury and shikimic acid accumulation. Shikimic acid accumulation increased when glyphosate was applied at 107 g ha<sup>-1</sup> or greater. U.S. No.1 potato yield was reduced by 46% and 84% relative to the untreated control (55 and 76 T/ha) when glyphosate was applied at 107 g ha<sup>-1</sup> to plants in the hooking stage at Ontario and Paterson, respectively. Tuber yields at both sites were lowest when glyphosate was applied at hooking and tuber initiation stages.

**Nomenclature:** Glyphosate; potato, *Solanum tuberosum* L. 'Ranger Russet', SOLTU.

**Key words:** Potato hooking stage, glyphosate application timing, shikimic acid.

En 2008 se realizaron estudios de campo en Ontario, OR y Paterson, WA para determinar el efecto de la diseminación o rociado no intencional de glifosato en el daño de papa 'Ranger Russet', en la acumulación del ácido shikímico y en el rendimiento del tubérculo. El glifosato fue aplicado a 8.5, 54, 107, 215 y 423 g ea ha<sup>-1</sup>, lo que corresponde a 0.01, 0.064, 0.126, 0.254 y 0.5 de la dosis más baja recomendada (846 g ha<sup>-1</sup>) en una sola aplicación para maíz y remolacha azucarera resistentes a glifosato. El glifosato fue aplicado cuando las plantas de papa tenían: 10 cm de altura, iniciación temprana del tubérculo, iniciación del tubérculo y etapa de ensanchamiento del tubérculo. El mayor daño foliar fue registrado cuando el glifosato se aplicó a una dosis mayor o igual a 54 g ha<sup>-1</sup> y cuando las plantas estaban en la etapa de iniciación temprana del tubérculo. El menor daño foliar fue observado cuando el glifosato se aplicó a las plantas en la etapa de ensanchamiento del tubérculo. La dosis I<sub>50</sub> de glifosato a 42 DAT, fue estimada a ser 167 g ha<sup>-1</sup> para papas rociadas en la etapa de iniciación temprana del tubérculo. Las dosis de glifosato que resultaron en un daño del 50% para plantas rociadas en las etapas de iniciación del tubérculo, de 10 cm de altura y en la etapa de ensanchamiento del tubérculo fueron 129%, 338% y 438% mayores que en la etapa de iniciación temprana del tubérculo. El rendimiento de la papa U.S. No. 1 fue inversamente relacionado al daño de la enredadera y a la acumulación de ácido shikímico. La acumulación de ácido shikímico aumentó cuando el glifosato fue aplicado igual o mayor a 107 g ha<sup>-1</sup>. El rendimiento de la papa U.S. No. 1 se redujo en 46 y 84% en relación con el testigo no tratado (55 y 76 T/ha) cuando el glifosato fue aplicado en 107 g ha<sup>-1</sup> a plantas en la etapa de iniciación temprana del tubérculo en Ontario y Paterson, respectivamente. Los rendimientos del tubérculo en ambos sitios fueron los más bajos cuando el glifosato se aplicó en las etapas de iniciación temprana del tubérculo y de iniciación de tubérculo.

Glyphosate is a nonselective postemergence herbicide used to control annual and perennial weeds in reduced tillage systems and in herbicide-resistant crops. Since glyphosate-resistant crops were launched in 1996, glyphosate use has been increasing in the United States. Nationally, about 12 million kg of glyphosate active ingredient were used on corn alone in 2005 (NASS 2005). In Eastern Oregon and central Washington, glyphosate is applied directly to tolerant crops, including alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), and sugar beet (*Beta vulgaris* L.). Glyphosate is also used pre-plant

as a burn-down weed treatment early in the season before planting onion (*Allium cepa* L.), pinto beans (*Phaseolus vulgaris* L.), and other crops. Noncrop uses include applications to control weeds along ditch banks and fence lines throughout summer. The proximity of fields planted to glyphosate-resistant and -susceptible crops increases the potential for off-target injury. In addition, glyphosate application timing for weed control in resistant crops coincides with the active growth stage for potatoes—a time when plants are most susceptible to off-target movement of herbicides (Hurst 1982; Snipes et al. 1991).

Glyphosate-resistant sugar beets were launched in 2008 in the Pacific Northwestern states, and will probably be remembered as the most rapidly adopted herbicide-resistant crop technology event ever. The launching year resulted in an estimated 99% use of glyphosate-resistant sugar beets in Oregon and Idaho (J. Felix, personal observation). Large

DOI: 10.1614/WT-D-11-00001.1

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Table 1. Soil properties, planting date, and spray timing for simulated glyphosate drift study at Ontario, OR, and Paterson, WA, 2008.

	Ontario, OR	Paterson, WA
Soil type	Owyhee silt loam <sup>a</sup>	Quincy sand <sup>b</sup>
pH	6.9	7.0
Organic matter (%)	1.87	0.4
Soil texture		
Sand (%)	17	92
Silt (%)	65	5
Clay (%)	18	3
Spraying timings		
Potato plants at 5–10-cm height	May 26	May 9
Potato plants at hooking stage	June 5	May 1
Potato plants at tuber initiation	June 13	May 27
Potato plants at tuber bulking stage	July 1	June 13

<sup>a</sup> Owyhee silt loam (coarse-silty, mixed, mesic, xerollic camborthid).

<sup>b</sup> Quincy sand (mixed, mesic Xeric Torripsamments).

numbers of fields planted to glyphosate-resistant crops and multiple applications of glyphosate during the season increases the chance of accidental drift to susceptible crops. Herbicides that inhibit amino acid synthesis often reduce potato leaf size and internode length (Eberlein et al. 1997). New leaves often turn chlorotic and plant growth is greatly reduced. Even though the potato plants might appear to recover, tubers can have numerous growth cracks and folds, greatly reducing potato quality and yields. In addition to initial foliar damage, herbicide drift can reduce tuber quality in potatoes (Eberlein and Guttieri 1994).

Off-target movement of herbicides during application can cause considerable injury to susceptible plants. The degree of damage to crops from drift depends on many factors, including plant species, growth stage, environmental conditions, herbicide formulation, droplet size, and spray height above the target (Bode 1987; Deeds et al. 2006; Hanks 1995; Masiunas and Weller 1988; Miller 1993; Smid and Hiller 1981). Additionally, small spray droplets ( $\leq 150 \mu\text{m}$  diam), which result from using smaller orifice nozzles and higher operating pressures, are more subject to drift (Yates et al. 1985). Research reports have shown that downwind drift deposits from unshielded sprayers can be less than 1% to as much as 16% of the target dose (Bode 1987; Maybank et al. 1978). However, even these low herbicide doses can severely injure susceptible crops (Al-Khatib et al. 2003). The objectives of these studies were to determine visual injury and yield reduction of potato tubers in response to simulated glyphosate drift when applied at different doses and potato growth stages.

## Materials and Methods

Field studies were conducted in 2008 at the Malheur Experiment Station near Ontario, OR and Paterson, WA to evaluate the response of potato to simulated glyphosate drift applied at different doses and crop growth stages. Soil characteristics for each site are presented in Table 1. Primary tillage followed local recommendations for potato production. Similarly, fertilization, other pest control, and irrigation followed standard potato production practices in the western

United States (Strand 2006). Potato variety 'Ranger Russet' was planted on April 23 and March 19, 2008 with a seed spacing of 22.5 cm in rows spaced 90 and 86 cm apart, at Ontario and Paterson, respectively. Potato rows were harrowed and reilled (standard grower practice in Pacific Northwest) just prior to potato emergence. Experiments were established in a split-plot design with treatments arranged in randomized complete block with four replications. Herbicide application timing formed the main plots, while isopropylamine salt of glyphosate<sup>1</sup> doses were randomly assigned to split-plots. Plots were 2.7 m wide by 9.1 m long and 2.6 m wide by 11 m long at Ontario and Paterson site, respectively.

Glyphosate application timings were made when potato plants were at 10 cm height, hooking stage (stolon swelling or early tuber initiation), tuber initiation, and at potato tuber bulking stage. Dates corresponding to respective application timings are presented in Table 1. The application dates were chosen to coincide with application of glyphosate to tolerant corn and sugar beet. Glyphosate doses evaluated were 8.5, 54, 107, 215, and 423 g ha<sup>-1</sup>, which corresponds to 0.01, 0.064, 0.126, 0.254, and 0.5, respectively, of the lowest recommended (846 g ha<sup>-1</sup>) single application rate for glyphosate in transgenic corn and sugar beet (Anonymous 2007). All treatments included ammonium sulfate<sup>2</sup> at 2.5% v/v and an untreated control was also included. Herbicides were applied in a total spray volume of 187 L ha<sup>-1</sup> on the dates indicated in Table 1. Herbicide was applied using a backpack compressed-CO<sub>2</sub> sprayer<sup>3</sup> with a boom equipped with six 8002 EVS and 8002 XR<sup>4</sup> flat-fan nozzles operated at 241 and 186 kPa at Ontario and Paterson, respectively. Plots were sprayed PRE with a tank mixture of S-metolachlor and pendimethalin at 1,060 and 1,420 g ai ha<sup>-1</sup>, respectively, to help maintain weed-free conditions for the duration of the study, supplemented by periodic hand weeding.

A modified spectrophotometric method was used to quantify shikimic acid accumulation in the potato plant tissue (Pline et al. 2001; Singh and Shaner 1998). At 7 d after treatment (DAT), 10 random plants within a plot were selected and a single leaf disk collected from each plant and pooled. A 7-mm-diam hole punch<sup>5</sup> was used to collect each sample adjacent to the leaf midrib. The accumulation of shikimic acid in leaves of glyphosate-treated plants has been reported to be transitory in nature, with the highest amount recorded at 7 DAT in tobacco (*Nicotiana tabacum* L.) (Burke et al. 2005), sunflower (*Helianthus annuus* L.), proso millet (*Panicum miliaceum* L.), wheat (*Triticum aestivum* L.) (Henry et al. 2007) and at 4 DAT in corn and soybean [*Glycine max* (L.) Merr.] (Henry et al. 2005). Leaf samples were harvested in order, from zero glyphosate dose to highest dose to avoid cross contamination. The hole punch was rinsed in deionized water after harvesting each plot. The leaf samples immediately were placed in a 1.5-ml microcentrifuge tube containing 0.5 ml of 0.01 M H<sub>2</sub>SO<sub>4</sub> and transported to the laboratory in an iced cooler at 4 C. Samples from each site were packaged with dry ice and shipped overnight to Pullman, WA for shikimic acid determination. On arrival in Pullman WA, the plant material was stored at -40 C until used for extraction. Extraction techniques followed those of Pline et al. (2002) and Singh and Shaner (1998). Plant

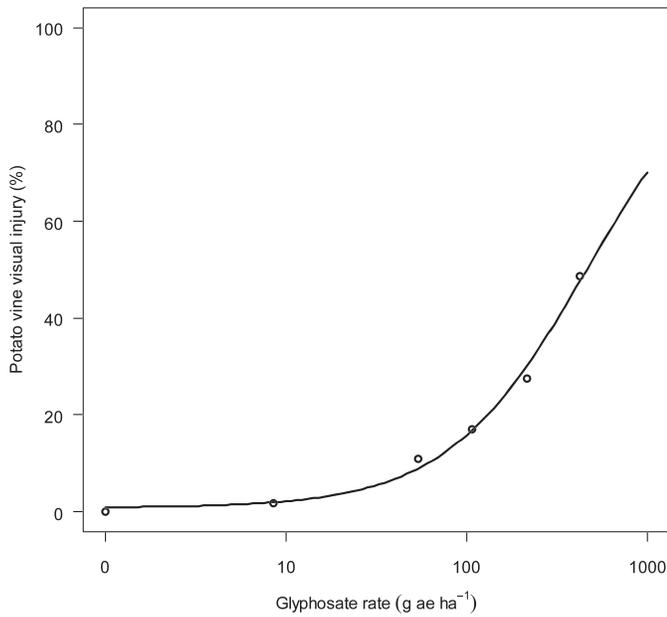


Figure 1. Regression line (Equation 1) was fit to combined site and glyphosate application timing for potato foliar visual injury 7 d after glyphosate application in 2008 field studies at Ontario, OR and Paterson, WA. Regression parameter estimates for glyphosate doses required to produce 5%, 10%, and 90% injury are presented in Table 2. Values in the x-axis are in log scale.

material was ground in the microcentrifuge vial in which it was collected. After maceration, 0.25 ml of 0.4 M NaCO<sub>3</sub> was added, the extract was agitated, and then centrifuged at 10,000 × *g* for 4 min. The extract was stored at -40 C or analyzed immediately. Two 20-μl aliquots of each sample were mixed in 0.5 ml of 1% wt/v periodic acid in separate microcentrifuge vials and allowed to oxidize. After 3 h, 0.5 ml of 1 N NaOH was added to the sample vial and 0.5 ml of deionized water was added to the sample standard vial. An additional 0.3 ml of 0.1 M glycine was added to each vial and agitated. The optical density of each solution was measured at 380 nm. Sample standard values were subtracted from sample values to account for any absorbance caused by plant material, and this standardized value was used to compute the milligrams of shikimic acid per 10 leaf disks of fresh weight of potato per plot based on a standard curve (Pline et al. 2002; Singh and Shaner 1998). Standard curves were developed by using pure shikimic acid standard of known concentrations.

Potato plant injury was visually assessed on a scale of 0 to 100% (where 0 = no injury and 100% = crop death) at 7, 21, and 42 DAT at each site. Potato yield was determined on September 19 and September 11, 2008 at Ontario and Paterson, respectively, by weighing tubers harvested with the use of a mechanical harvester from 6 m of the center row. Tubers from each plot were graded by size and quality according to U.S. Department of Agriculture grading standards (Anonymous 1991).

Nontransformed data were subjected to ANOVA with the use of PROC GLM procedure in SAS.<sup>6</sup> Type III statistics were used to test for significant differences ( $P \leq 0.05$ ) of

sites, glyphosate dose, application timings, and their interactions for visual plant injury, potato yield, and shikimic acid accumulation variables. All the data were subjected to a normality test. Because analysis of square root-transformed data did not change the results of ANOVA, the nontransformed data were used in the final analysis. Data were pooled across sites or timings when no significant effects for site, timing, or site-by-timing interactions were detected. Regression of potato plant injury ratings, yield, and shikimic acid accumulation over herbicide dose was done using a four-parameter log-logistic model as described by Seefeldt et al. (1995) as indicated below:

$$Y = C + \{D - C / 1 + \exp[b(\log x - \log e)]\} \quad [1]$$

where  $Y$  is the response (e.g., percent of potato injury),  $C$  is the lower limit,  $D$  is the upper limit,  $b$  is the slope of the line,  $x$  is the herbicide dose, and  $e$  is the dose resulting in a 50% response (e.g., 50% injury, which is also known as effective dose 50 [ $I_{50}$ ]). Analysis of the dose-response curves and ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>90</sub> values were determined using the open-source statistical software, R<sup>®</sup> 2.7.2, and the drc package as described by Knezevic et al. (2007). Comparison of means was performed with the use of Fisher's protected LSD test at a  $P \leq 0.05$ .

## Results and Discussion

**Plant Injury.** The data for visual foliar injury evaluations at 7 DAT were combined across sites and application timing because the ANOVA indicated no significant difference between sites, glyphosate application timing, or their interactions with glyphosate dose (Figure 1). Potato foliar injury was characterized by chlorosis of the newest leaves that increased in severity with glyphosate dose. There were few or no injury symptoms when glyphosate was applied at 8.5 g ha<sup>-1</sup>. Potato foliar injury at 7 DAT was related directly to glyphosate dose. Foliar injury ranged from 2% for plants sprayed with 8.5 g ha<sup>-1</sup> to 49% for those sprayed with 423 g ha<sup>-1</sup>. The calculated  $I_{50}$  at 7 DAT was 468.3 g ha<sup>-1</sup> (Table 2). Corresponding doses calculated for ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>90</sub> were 30.5, 60.9, and 3,598.1 g ha<sup>-1</sup>, respectively.

The use of nonlinear regression models to determine effective doses for plant injury at a predetermined level has been described as a functional approach by Berti et al. (1996) and has been recommended by Knezevic et al. (2002, 2007) for weed and crop injury studies. Lower visual injury at 7 DAT compared to later ratings likely can be attributed to the slow action of glyphosate in treated plants (Stoller et al. 1975). Also, leaves are considered a sink during early potato growth stages before reserves from the seed tuber are exhausted. The  $I_{50}$  and the shape of the dose response curve can change with time after treatment (Burke et al. 2005). Partially injured plants can compensate for injury with time, resulting in minimal effects on yield quantity (Seefeldt et al. 1995). Glyphosate sprayed to plants at 10 cm height tended to induce production of new shoots from the tuber seed piece, which did not display injury symptoms but were delayed in development compared to untreated controls.

Table 2. Regression parameter estimates and glyphosate dose ( $\text{g ae ha}^{-1}$ ) to result in 5, 10, and 90% potato visual injury and accumulation of shikimic acid ( $\text{ED}_{5,10,90}$  [ $\pm \text{SE}$ ]) based on visual ratings at 7, 21, and 42 DAT and accumulation of shikimic acid at 7 DAT.<sup>a</sup>

Variable	Timing	Regression parameters ( $\pm \text{SE}$ )						
		<i>b</i>	<i>C</i>	<i>D</i>	<i>I</i> <sub>50</sub>	ED <sub>5</sub> ( $\pm \text{SE}$ )	ED <sub>10</sub> ( $\pm \text{SE}$ )	ED <sub>90</sub> ( $\pm \text{SE}$ )
Injury 7 DAT	Average	-1.08 (0.13)	0.02 (1.69)	100	468.3 (36.9)	30.5 (8.8)	60.9 (12.9)	3,598.1 (101.9)
Injury 21 DAT (combined)	10-cm height	-1.1 (0.40)	-0.005 (2.4)	105.2 (55.1)	320.0 (3.1)	27.9 (99.1)	44.1 (19.4)	2,321.8 (104.2)
	Hooking	-2.7 (0.38)	-0.045 (2.0)	91.3 (2.8)	80.3 (4.1)	22.5 (4.1)	36.6 (5.3)	176.1 (45.8)
	Initiation	-2.3 (0.35)	0.018 (1.9)	88.7 (6.2)	156.4 (14.9)	44.3 (8.9)	61.0 (9.1)	401.1 (65.3)
	Bulking	-0.83 (0.25)	0.022 (2.4)	296.9 (60.8)	4,285.5 (1,425.4)	122.9 (204.3)	302.8 (370.0)	8,383.3 (327.8)
Injury 42 DAT (combined)	10-cm height	-1.5 (1.42)	-0.003 (3.7)	120.8 (475.7)	731.4 (3,168.9)	98.4 (286.9)	163.8 (584.3)	3,266.4 (2,539.2)
	Hooking	-1.8 (0.58)	-0.006 (3.8)	96.8 (19.8)	167.0 (51.1)	32.9 (17.7)	49.7 (19.4)	561.0 (755.8)
	Initiation	-1.9 (1.09)	-0.003 (3.9)	70.8 (28.9)	214.8 (127.8)	46.3 (14.5)	68.4 (14.8)	674.5 (372.7)
	Bulking	-1.2 (2.16)	0.001 (4.2)	55.1 (268.8)	564.8 (4,119.1)	48.0 (143.8)	89.8 (324.3)	3,553.1 (2,740.9)
Shikimic acid (Ontario, OR)	10-cm height	-2.3 (0.33)	1.4 (1.9)	99.9 (3.9)	379.7 (20.3)	107.1 (75.6)	147.6 (78.1)	976.9 (502.9)
	Hooking	-2.8 (0.30)	1.6 (2.1)	99.8 (3.8)	138.7 (6.3)	48.9 (30.8)	63.7 (28.7)	301.9 (195.5)
	Initiation	-4.5 (1.96)	3.5 (1.7)	98.6 (4.5)	454.5 (20.2)	235.6 (11.1)	278.4 (10.2)	742.0 (25.6)
	Bulking	-4.7 (1.28)	2.2 (1.7)	98.8 (3.2)	410.6 (12.3)	219.6 (13.4)	257.4 (14.2)	655.0 (41.1)
Shikimic acid (Paterson, WA)	Hooking	-2.9 (0.28)	1.9 (1.4)	98.8 (2.8)	328.9 (11.4)	118.4 (70.2)	153.5 (102.9)	705.3 (817.7)
	Initiation	-2.0 (0.99)	1.1 (2.1)	99.5 (3.4)	835.8 (299.5)	193.2 (100.3)	280.2 (934.7)	2493.1 (191.2)
	Bulking	-3.5 (10.11)	1.1 (2.0)	19.8 (3.2)	874.3 (181.1)	376.6 (26.3)	466.3 (24.8)	1,639.4 (83.1)
	Yield (Ontario)	10-cm height	1.2 (0.26)	0.060 (4.4)	99.3 (4.4)	287.6 (44.9)	23.9 (25.9)	44.9 (36.5)
Yield (Ontario)	Hooking	1.3 (0.27)	0.010 (4.5)	94.7 (4.5)	164.3 (24.0)	17.6 (12.7)	30.9 (16.7)	871.6 (477.3)
	Initiation	2.2 (0.43)	-0.001 (3.9)	102.9 (3.9)	114.1 (10.1)	30.0 (9.0)	42.1 (11.4)	309.2 (64.8)
	Bulking	2.5 (0.51)	-0.001 (3.7)	100.6 (3.7)	204.2 (17.2)	63.9 (15.7)	85.8 (20.1)	485.8 (117.5)
	Yield (Paterson)	10-cm height	1.3 (0.36)	-0.03 (3.5)	96.9 (3.5)	480.3 (77.1)	46.8 (45.3)	84.5 (61.2)
Hooking		2.5 (0.53)	-0.04 (3.3)	96.8 (3.3)	56.9 (4.5)	17.5 (4.0)	23.6 (4.4)	137.4 (21.3)
Initiation		2.2 (0.41)	-0.03 (3.3)	102.3 (3.3)	71.6 (5.1)	19.2 (6.6)	26.8 (7.1)	191.3 (40.2)
Bulking		3.1 (0.52)	-0.03 (2.9)	98.5 (2.9)	160.7 (9.8)	62.9 (27.3)	79.8 (35.4)	323.4 (170.2)

<sup>a</sup> Abbreviations: *b*, slope of line; *C*, lower limit; *I*<sub>50</sub>, the glyphosate dose needed to cause a 50% visual injury; ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>90</sub>, are the glyphosate doses needed to cause 5%, 10%, and 90%, respectively, foliar injury or tuber reduction; DAT, d after treatment.

ANOVA indicated no significant difference between sites for the potato visual foliar injury data at 21 DAT, but there was a glyphosate-by-timing interaction (Figure 2). Injury severity increased with glyphosate dose, regardless of the application timing. The greatest visual injury was observed when glyphosate was applied at a dose of  $54 \text{ g ha}^{-1}$  or greater and potato plants were at the hooking stage. The lowest vine injury was observed when glyphosate was applied to potato plants at the bulking stage. The estimated *I*<sub>50</sub> glyphosate dose at 21 DAT was lowest at hooking stage ( $80.3 \text{ g ha}^{-1}$ ) followed by tuber initiation ( $156.4 \text{ g ha}^{-1}$ ) (Table 2). The estimated glyphosate dose to result in 50% injury for potato plants sprayed at 10-cm height and bulking stage was 3.99 and 53.4 times greater than hooking stage (Table 2). Similarly, the ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>90</sub> (glyphosate dose to result in 5, 10, and 90% visual injury) at 21 DAT were lowest when glyphosate was applied at potato hooking stage. Similar results were observed for injury at 42 DAT (Figure 3). The *I*<sub>50</sub> glyphosate dose at 42 DAT was estimated to be  $167 \text{ g ha}^{-1}$  for potatoes sprayed at the hooking stage (Table 2). The corresponding glyphosate dose to result in 50% injury for potatoes sprayed at tuber initiation, 10-cm height, and bulking stages were 129%, 338%, and 438% greater than hooking stage. The higher glyphosate dose required to elicit 50% injury at tuber initiation and bulking stages was directly related to the size of potato plants at the time of application. Similarly, the ED<sub>5</sub>,

ED<sub>10</sub>, and ED<sub>90</sub> glyphosate dose at 42 DAT was lowest for plants sprayed at hooking and tuber initiation (Table 2). Increased injury in relation to glyphosate dose in simulated drift studies has been reported in other crops, including sorghum (Al-Khatib et al. 2003), tobacco (Burke et al. (2005), and rice (*Oryza sativa* L.) (Ellis et al. 2003; Koger et al. 2005). Also, Masiunas and Weller (1988) observed increased potato injury with glyphosate applied at field use doses.

**Shikimic Acid Accumulation.** ANOVA indicated a difference between sites for shikimic acid accumulation in potato plants at 7 DAT; therefore, the data are presented separately (Figure 4). Burke et al. (2005) reported that shikimic acid accumulation peaked at 7 DAT in tobacco. Similarly, Buehring et al. (2003) reported peak shikimic acid accumulation in corn at 5 DAT. Consequently, the samples for shikimic acid accumulation were sampled only at 7 DAT at both sites. Shikimic acid accumulation in potato plants increased with increasing glyphosate dose at each site. Shikimic acid accumulation increased when glyphosate was applied at  $107 \text{ g ha}^{-1}$  or greater (Figure 4A). Potato plants sprayed at the hooking stage had the greatest accumulation, and required a lower glyphosate dose to trigger shikimic acid accumulation at both sites. The *I*<sub>50</sub> dose for plants treated at hooking stage was  $138.7 \text{ g ha}^{-1}$  at Ontario (Table 2). The corresponding *I*<sub>50</sub> dose for plants sprayed at 10-cm height,

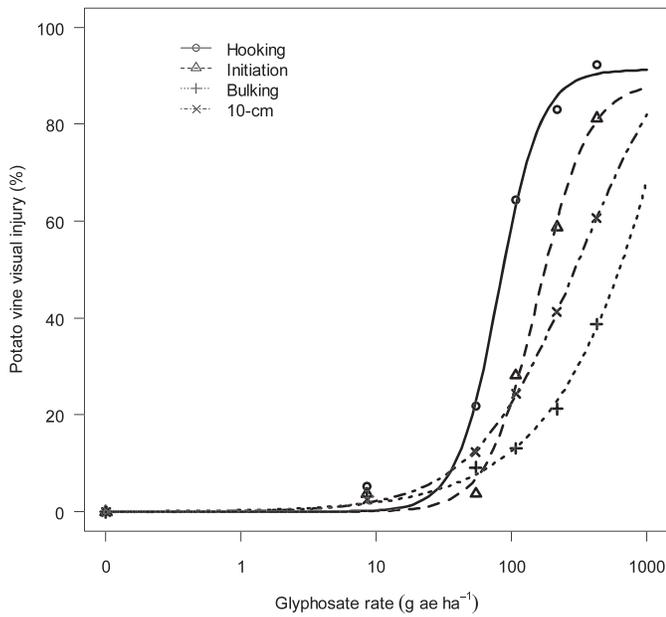


Figure 2. Regression lines (Equation 1) were fit to glyphosate dose and application timing for potato foliar visual injury 21 d after glyphosate application in 2008 field studies at Ontario, OR and Paterson, WA. Regression parameter estimates for glyphosate doses required to produce 5%, 10%, and 90% injury are presented in Table 2. Values in the x-axis are in log scale.

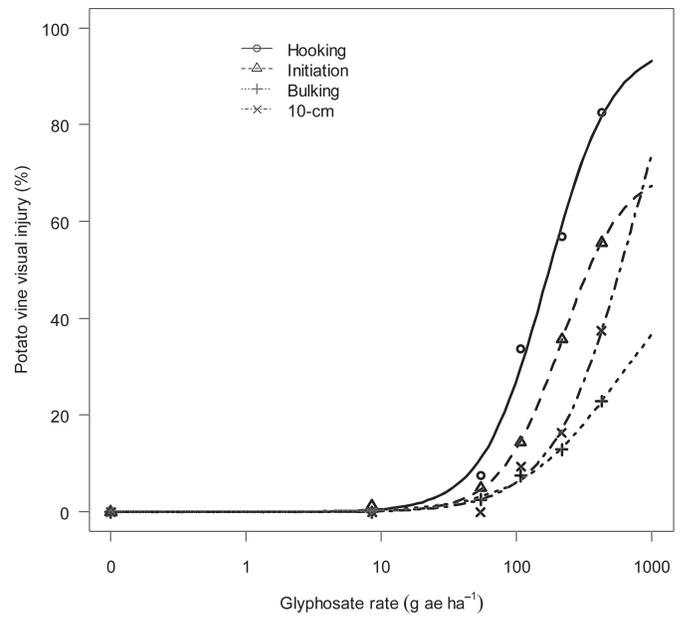


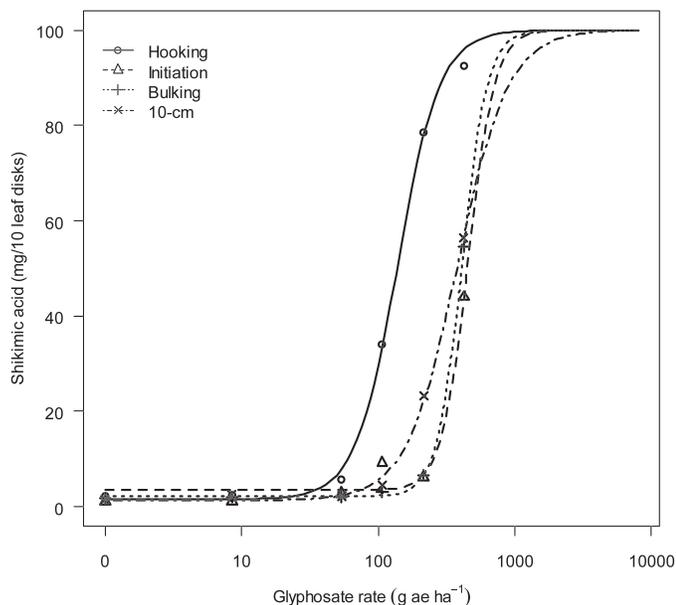
Figure 3. Regression lines (Equation 1) were fit to glyphosate dose and application timing for potato foliar visual injury 42 d after glyphosate application in 2008 field studies at Ontario, OR and Paterson, WA. Regression parameter estimates for glyphosate doses required to produce 5%, 10%, and 90% injury are presented in Table 2. Values in the x-axis are in log scale.

tuber initiation, and bulking were 379.7, 454.5, and 410.6 g ha<sup>-1</sup>, respectively. The ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>90</sub> for plants sprayed at hooking stage at Ontario was estimated to be 48.9, 63.7, and 301.9 g ha<sup>-1</sup>, respectively. The lower glyphosate doses required to trigger accumulation of shikimic acid at potato hooking suggest greater potato sensitivity at this stage compared to plants sprayed at tuber initiation, bulking, or 10-cm height stage. Hooking stage marks rapid plant growth and a transition from vegetative to tubers becoming a major sink for photoassimilates. As a result, the majority of glyphosate drift at the hooking stage is translocated rapidly to newly formed leaves and tubers, culminating in greater injury. At the Paterson site, application of glyphosate at the hooking stage elicited the same response as that at Ontario (Figure 4B). No samples were taken to determine shikimic acid accumulation at the 10-cm height stage at Paterson. The *I*<sub>50</sub> dose for shikimic acid accumulation for potatoes sprayed at hooking stage was 328.9 g ha<sup>-1</sup> (Table 2). The *I*<sub>50</sub> dose values for potatoes sprayed at tuber initiation and bulking stage were 835.8 and 874.3 g ha<sup>-1</sup>, respectively. The ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>90</sub> for plants sprayed at hooking stage at Paterson was 118.4, 153.5, and 705.3 g ha<sup>-1</sup>, respectively. Significantly greater amounts of glyphosate were needed to elicit the same response for plants sprayed at tuber initiation and bulking stage than at the hooking stage (Table 2). The estimated *I*<sub>50</sub> for shikimic acid accumulation at potato hooking stage (138.7 g ha<sup>-1</sup>) was greater than that estimated for U.S. No.1 tuber yield 56.9 g ha<sup>-1</sup> but lower for visual estimates of injury at 42 DAT (167 g ha<sup>-1</sup>). These results suggest that less glyphosate is needed to reduce tuber yield compared to the amount needed to elicit visual injury.

**Yield.** There were site-by-timing and site-by-glyphosate interactions for the U.S. no.1 potato tuber yield; therefore, the data are presented separately for each site (Figure 5). Symptoms on potato tubers affected by glyphosate were characterized by growth cracks, folds, “elephant hide,” malformation, and small-sized tubers. Tuber injury severity and shikimic acid accumulation increased with the increase in glyphosate dose.

Potato tuber yields were directly related to level of foliar injury observed earlier in the season (Figures 1–3) and shikimic acid accumulation (Figure 4). As potato vine injury and shikimic acid accumulation increased with glyphosate dose and timing, potato yield decreased accordingly. Tuber yields at both sites were lowest when glyphosate was applied at hooking and tuber initiation stages. At Ontario, U.S. No.1 tuber yields for plants treated with 107 g ha<sup>-1</sup> glyphosate at hooking and tuber initiation stages were only 54% and 52% of the nontreated (54 and 52 T ha<sup>-1</sup>), respectively. The *I*<sub>50</sub> dose for potato yield at Ontario when glyphosate was applied at hooking, tuber initiation, bulking and 10-cm height were estimated to be 164.3, 114.1, 204.2, and 287.6 g ha<sup>-1</sup>, respectively (Table 2). The estimated dose required to reduce potato tuber yield by 5%, 10%, and 90% when glyphosate drift occurred at the potato hooking stage were 17.6, 30.9, and 871.6 g ha<sup>-1</sup>, respectively, at Ontario. At Paterson, U.S. No.1 tuber yield was reduced to 16% and 23% of the nontreated control (76 and 72 T ha<sup>-1</sup>) when glyphosate was applied at 107 g ha<sup>-1</sup> to plants in the hooking and tuber initiation stages, respectively. The *I*<sub>50</sub> dose for potato tuber yield was 56.9 g ha<sup>-1</sup> for the hooking timing (Table 2). The corresponding *I*<sub>50</sub> doses for sprays at tuber initiation, bulking, and 10-cm height timings at Paterson were 126%, 282%, and

A. Shikimic acid accumulation in leaves at Ontario



B. Shikimic acid accumulation in leaves at Paterson

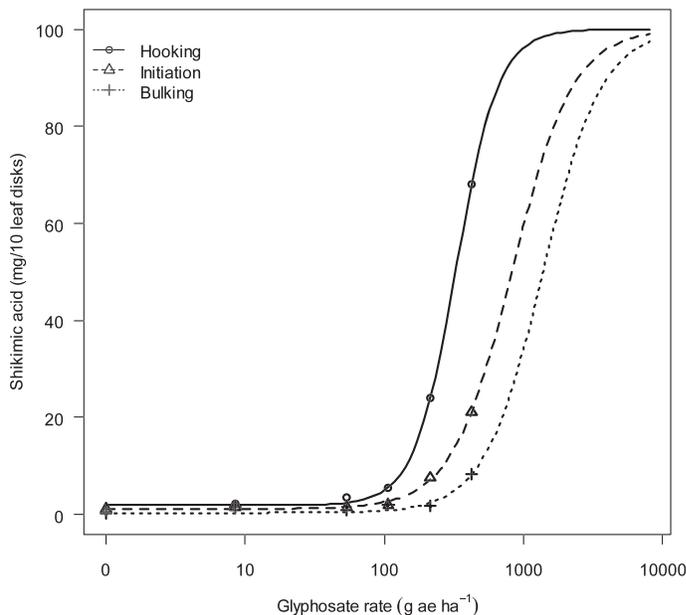
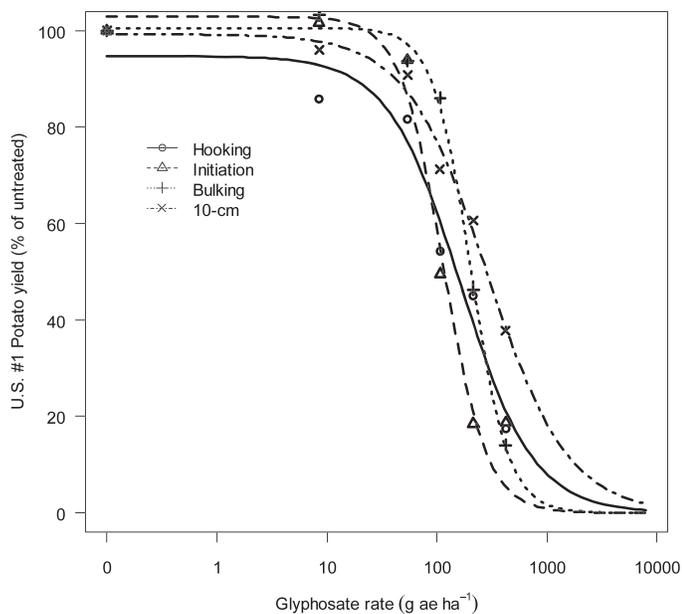


Figure 4. Regression lines (Equation 1) were fit to shikimic acid accumulation 7 d after different glyphosate doses and application timing in field studies at Ontario, OR (4A) and Paterson, WA (4B) in 2008. Regression parameter estimates for glyphosate doses required to produce 5%, 10%, and 90% shikimic acid accumulation are presented in Table 2. Values in the x-axis are in log scale.

844%, respectively, greater than that at the hooking stage. The ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>90</sub> for potatoes sprayed at the hooking stage at Paterson were 17.5, 23.6, and 137.4 g ha<sup>-1</sup>, respectively. Auwarter and Hatterman-Valenti (2006) and

Hatterman-Valenti and Auwarter (2009) reported reduced marketable potato tuber yield when simulated glyphosate drift with 280 g ha<sup>-1</sup> happened at tuber initiation and early bulking stages. Similarly, Pfleger et al. (2008) reported

A. Potato yield at Ontario, OR



B. Potato yield at Paterson, WA

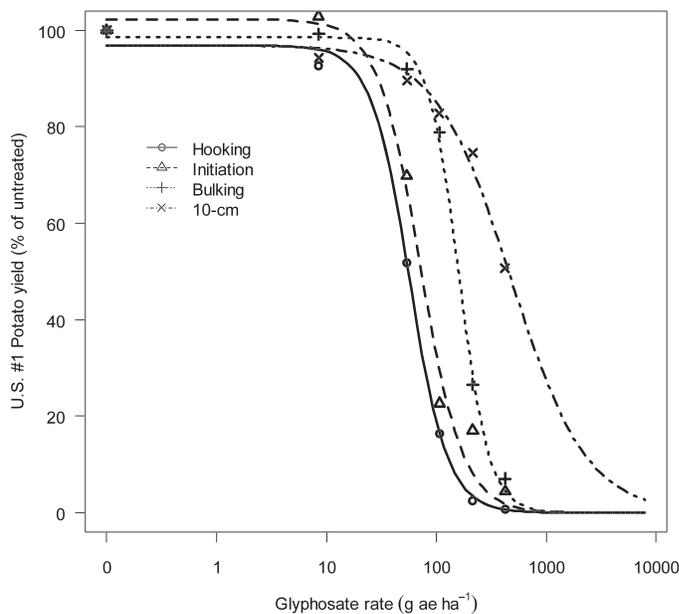


Figure 5. Regression lines (Equation 1) were fit to glyphosate dose and application timing for potato U.S. No.1 tuber yield in response to glyphosate and application timing for field studies at Ontario, OR (5A) and Paterson, WA (5B) in 2008. Regression parameter estimates for glyphosate doses required to produce 5%, 10%, and 90% yield reduction are presented in Table 2. Values in the x-axis are in log scale.

reduced U.S. No.1 potato tuber yield with 15 g ha<sup>-1</sup> glyphosate applied 14 d after potato emergence.

Potato plant injury was characterized by stunting (for the 10-cm application timing) and general interveinal chlorosis of newest leaves. Leaf chlorosis was evident at rates starting 54 g ha<sup>-1</sup> and higher. Foliar injury was greater at 21 and 42 DAT than at 7 DAT, typical of the slow activity associated with glyphosate (Stoller et al. 1975). Increased injury with time also has been reported in tobacco by Burke et al. (2005). Newly emerged shoots treated at the 10-cm plant height were stunted and never recovered. However, new shoots emerged and grew normally. Seefeldt et al. (1995) reported that partially injured plants can compensate vegetative growth with time, resulting in minimal effects on yield quantity at the end of the season. Potato tuber yield was reduced by a glyphosate dose of 54 g ha<sup>-1</sup> or greater at each site. Potato tuber yield was less impacted for plants treated at the 10-cm height with glyphosate dose at 215 g ha<sup>-1</sup> and above, suggesting that new shoots established after the original ones were killed compensated the final yield. In addition to the deleterious effects of glyphosate on tuber yield and quality, glyphosate drift also has potential to negatively affect sprouting of daughter tubers derived from seed tuber production fields (Smid and Hiller 1981).

Sink regulation of photosynthesis is a well-accepted concept, possibly explaining the coordination of assimilate production and consumption (Stitt et al. 1990). In tobacco and potato, the photosynthetic capacity of source leaves is under developmental and environmental control (Miller et al. 1997). Tuberization in potato is a complex process involving anatomical, hormonal, and biochemical changes, leading to the differentiation of a lateral shoot (the stolon) into a vegetative storage organ (the tuber) (Mohammad-Reza et al. 2000). Starting at the hooking stage, most of the sucrose produced is shunted into tubers, which serve as storage organs in potato. This might explain why the greatest injury was observed at the hooking stage than at any other glyphosate application timing.

In summary, the results indicated greater injury to potato plants receiving a glyphosate dose of 54 g ha<sup>-1</sup> or greater during the hooking or tuber initiation stages. Most growers would not be able to ascertain injury from low glyphosate dose typical of accidental drift. However, the results indicated that shikimic acid accumulates and can be detected in plants receiving very low glyphosate doses that might not show injury symptoms. Using a shikimic acid assay would allow growers to confirm glyphosate drift if they observe or suspect a drift situation even when no foliar symptoms develop. The fast and inexpensive procedure to extract and determine shikimic acid accumulation in plants (Shaner et al. 2005) could also help growers positively confirm glyphosate drift well before visible symptoms are apparent. Because the assay requires sophisticated equipments, growers will have to seek help from capable Agricultural laboratories. Growers and farm managers could then decide whether or not to maintain potato plantings without risking reduced marketable yield and quality. In typical drift cases there is a concentration gradient down wind, with higher amounts deposited along the point of field entry or field margin. Growers can consult nearby weather stations and use the prevailing wind direction at the

time of application to guide them in sample collection for shikimic acid accumulation measurements.

## Sources of Materials

<sup>1</sup> Roundup Original Max<sup>®</sup> 4 EC, Monsanto Company, 800 North Lindburg Boulevard, St. Louis, MO 63167.

<sup>2</sup> Bronc<sup>®</sup>, a water-conditioning agent containing ammonium sulfate solution (41.75% of proprietary blend of ammonium sulfate, sodium alkyl aryl sulfonates, polycarboxylic acid, and silicone), marketed by Wilbur Ellis Company, 1801 Oakland Boulevard, Suite 210, Walnut Creek, CA 94596.

<sup>3</sup> CO<sub>2</sub> Sprayers Systems, Bellspray Inc., R&D Sprayers, P.O. Box 267, Opelousas, LA 70571.

<sup>4</sup> TeeJet 8002 EVS and 8002 XR flat-fan nozzle tips, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60188.

<sup>5</sup> 7-mm-diam, aluminum paper hole punch, McGill Incorporated, 131 E. Prairie St., Marengo, IL 60152.

<sup>6</sup> PROC GLM, Statistical Analysis Systems (SAS) software, Version 9.2. Statistical Analysis Systems Institute, Inc., P.O. Box 8000, Cary, NC 25712-8000.

## Acknowledgments

The authors would like to thank Joey Ishida, Encarnation Rivera, and Marc Seymour for their technical assistance in conducting the studies. We also would like to thank Dr. Stevan Knezevic for his help with the development of the R codes used to analyze the data with the log-logistic model.

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Received January 5, 2011, and approved July 27, 2011.