

## Response of Direct-Seeded Dry Bulb Onion to Simulated Glyphosate Drift with Variable Rates and Application Timings

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Field studies were conducted in 2011 at the Malheur Experiment Station, Ontario, OR and Prosser, WA to evaluate the effect of simulated glyphosate drift on direct-seeded dry bulb onion. Glyphosate was applied at 8.6, 25.8, 86, 290, 434, and 860 g ae ha<sup>-1</sup> when onion plants were at the flag-, two-, four-, and six-leaf stages. Onion foliar injury was directly related to the glyphosate dose and varied with application timing. Foliar injury at 7 d after treatment (DAT) ranged from 0 to 12% for glyphosate ≤ 25.8 g ha<sup>-1</sup>. Foliar injury increased at 21 DAT when glyphosate was applied ≥ 25.8 g ha<sup>-1</sup> to plants at the flag- and four-leaf stage, and ranged from 24 to 99%. The 50%-injury glyphosate dose at 21 DAT was lowest when onion was treated at the four-leaf and flag stages and was estimated to be 76.8 and 81 g ha<sup>-1</sup>, respectively. Onion injury severity increased when glyphosate was applied at ≥ 86 g ha<sup>-1</sup> and eventually resulted in plant death at 860 g ha<sup>-1</sup>. Foliar injury was inversely correlated to U.S. no. 1 onion yield. Onions displayed sensitivity to very low glyphosate doses especially at the four-leaf stage. Shikimic acid accumulation increased with the increase in glyphosate dose and was positively correlated with foliar injury and negatively correlated with plant height and onion yield.

**Nomenclature:** Glyphosate; onion, *Allium cepa* L. 'Vaquero'

**Key words:** Dry bulb onion, glyphosate application timing, shikimic acid.

Se realizaron estudios de campo en 2011 en la Estación Experimental Malheur, Ontario, OR y Prosser, WA para evaluar el efecto de la deriva simulada de glyphosate sobre el bulbo de cebolla seca de siembra directa. Se aplicó glyphosate a 8.6, 25.8, 86, 290, 434 y 860 g ae ha<sup>-1</sup> cuando las plantas de cebolla estaban en los estadios de hoja bandera, dos, cuatro y seis hojas. El daño foliar de la cebolla estuvo directamente relacionado a la dosis de glyphosate y varió con el momento de aplicación. El daño foliar a 7 días después del tratamiento (DAT) varió de 0 a 12% para glyphosate a ≤25.8 g ha<sup>-1</sup>. El daño foliar incrementó a 21 DAT cuando glyphosate se aplicó a ≥25.8 g ha<sup>-1</sup> a plantas en los estados de bandera y cuatro hojas y varió de 24 a 99%. La dosis más baja de 50% de daño de glyphosate a 21 DAT se observó cuando la cebolla fue tratada en los estados de cuatro hojas y hoja bandera y se estimó que fue 76.8 y 81 g ha<sup>-1</sup>, respectivamente. La severidad del daño de la cebolla aumentó cuando glyphosate se aplicó a ≥86 g ha<sup>-1</sup> y eventualmente resultó en la muerte de la planta a 860 g ha<sup>-1</sup>. El daño foliar estuvo inversamente correlacionado con el rendimiento de cebolla U.S. no. 1. La cebolla mostró sensibilidad a dosis muy bajas de glyphosate especialmente en el estado de cuatro hojas. La acumulación de shikimic acid aumentó con el incremento en la dosis de glyphosate y estuvo positivamente correlacionada con el daño foliar y negativamente correlacionada con la altura de la planta y el rendimiento de la cebolla.

Onion is an important crop in eastern Oregon, southwestern Idaho, and Washington. The onion production area in eastern Oregon and southwestern Idaho is within a radius of 48.3 km of Ontario, OR, on the Snake River plain and along the tributaries of the Snake River, a region frequently referred to as the Treasure Valley (Shock et al. 2005). Most storage onions in Washington are grown in the central part of the state in the Columbia Basin region in Grant, Franklin, Benton, and Adams counties. The leading county for non-storage bulb onion production in Washington is Walla Walla. Totals of 8,094 and 23,000 ha were planted to onion in the Treasure Valley and Washington State, respectively, in 2009, with a farm gate value of \$180 million and \$219 million, respectively (USDA 2009). However, the area planted to onion in each state can fluctuate greatly depending on growers' perceptions of market opportunities and prior year

prices. These onions are mostly produced from yellow long-day cultivars and are marketed fresh in August and September and from storage through April (Shock et al. 2000). The average dry bulb onion yield in 2009 in the Treasure Valley and Washington State was 81,000 and 68,000 kg ha<sup>-1</sup>, respectively (USDA 2009).

Onions are grown in fields interspersed with glyphosate-resistant alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), and sugar beet (*Beta vulgaris* L.). The proximity of fields planted to glyphosate-resistant and -susceptible crops increases the potential for off-target injury (Felix et al. 2011). Glyphosate application timing for weed control in resistant crops coincides with the active growth stage for onion, a time when plants are most susceptible to off-target movement of herbicides (Hurst 1982; Snipes et al. 1991). Large numbers of fields planted to glyphosate-resistant crops and multiple applications of glyphosate during the season increase the chance of accidental drift to susceptible crops (Felix et al. 2011). Herbicides that inhibit amino acid synthesis often reduce leaf size and internode length (Eberlein et al. 1997). Leaves of infected plants often turn chlorotic and plant growth is greatly reduced, if not completely killed.

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Glyphosate is a versatile herbicide used to control many annual and perennial weeds in herbicide-resistant crops. The use of glyphosate in the United States has steadily increased since resistant crops were introduced in 1996. About 29 million kg of different glyphosate salts were used on corn alone in 2010 in the United States (USDA 2010). Glyphosate is applied multiple times annually to resistant crops in the Treasure Valley and central Washington (Felix et al. 2011). Other uses include preplant or before crop emergence to control emerged weeds early in the season in onion and other crop fields. Noncrop uses include applications to control weeds along ditch banks and fence lines throughout summer.

Reports indicated that stray movement of herbicides during application can cause substantial injury to susceptible plants. Deeds et al. (2006) reported that off-target injury depends on several factors, including plant species, growth stage, environmental conditions, herbicide formulation, droplet size, and spray height above the target. Importantly, nozzle choice and sprayer operating pressure greatly affect spray droplets and may influence herbicide drift (Yates et al. 1985). Research results indicate that downwind drift deposits from nonshielded sprayers can be less than 1% to as much as 8% of the target dose (Bode 1987; Maybank et al. 1978). However, even low herbicide doses can severely injure susceptible crops (Al-Khatib et al. 2003). The objectives for our study were to determine crop injury, shikimic acid accumulation, and yield response of direct-seeded dry bulb onion to simulated glyphosate drift applied at different doses and onion growth stages.

## Materials and Methods

Field studies were conducted in 2011 at the Malheur Experiment Station near Ontario, OR and the Irrigated Agriculture Research and Extension Center near Prosser, WA to evaluate the response of dry bulb onion to simulated glyphosate drift applied at different doses and crop growth stages. Soil characteristics for each site are presented in Table 1. Primary tillage and other seedbed preparation activities followed local recommendations for onion production. Similarly, fertilization and other pest controls followed standard onion production practices as described by Shock et al. (2005). Yellow onion variety 'Vaquero' was planted on April 7, 2011 at Ontario, OR and April 15, 2011 at Prosser, WA in double rows using a customized planter equipped with John Deere Flexi Planter units and disc openers. The double rows on each 56-cm-wide bed were spaced 9.25 cm apart and seeds planted at 11 cm within each row (313,000 seeds ha<sup>-1</sup>). Experiments were established in a split-plot design with treatments arranged in a randomized complete block with four replications. Herbicide application timing formed the main plots, whereas isopropylamine salt of glyphosate (Roundup PowerMax, Monsanto Company, St. Louis, MO) doses were randomly assigned to split plots. Plots were 2.2 m wide by 9.1 m long at each site.

**Herbicide Application Timing.** Glyphosate was applied when onion plants were at the flag-, two-, four-, or six-leaf growth stages. Dates corresponding to the respective applica-

tion timings are presented in Table 1. The application dates were chosen to coincide with local application of glyphosate to resistant corn and sugar beet. Glyphosate doses evaluated were 8.6, 25.8, 86, 290, 434, and 860 g ha<sup>-1</sup>, which correspond to 0.01, 0.03, 0.1, 0.34, 0.5, and 1×, respectively, of the lowest recommended (860 g ha<sup>-1</sup>) single application dose for glyphosate in transgenic corn and sugar beet (Anonymous 2007). Glyphosate treatments included ammonium sulfate (Bronc Max, Wilbur-Ellis Company, Fresno, CA) at 2.5% v/v. A nontreated weed-free control was also included. Herbicides were applied in a total spray volume of 112 L ha<sup>-1</sup> on the dates indicated in Table 1. Herbicide treatments were applied using a pressurized CO<sub>2</sub> backpack sprayer (CO<sub>2</sub> Sprayers Systems, Bellspray Inc., R&D Sprayers, Opelousas, LA) with a boom equipped with four 8001 EVS and 8002 XR flat-fan nozzles (flat-fan nozzle tips, Spraying Systems Co., Wheaton, IL) operated at 241 and 186 kPa at Ontario and Prosser, respectively. Plots were sprayed PRE with pendimethalin (Prowl H<sub>2</sub>O, BASF Corporation, Research Triangle Park, NC) at 1,120 g ai ha<sup>-1</sup> on May 2 at Ontario, OR and April 26 at Prosser, WA to reduce weed emergence. Weed-free conditions for the duration of the study were achieved by supplemental weekly hand weeding of all plots at each site.

**Injury Evaluations and Crop Harvest.** Onion plant injury was visually assessed on a scale of 0 to 100% (where 0 = no injury and 100% = crop death) at 7, 21, and 35 DAT at each site. Plant height was determined at 35 DAT by randomly measuring 10 plants within the two center rows, each from the ground to the tip of the top-most fully expanded leaf. Onions were lifted on September 12 and left on the ground to cure for 7 d. Bulbs were harvested by hand from 4.6 m of the two center rows on September 19, 2011 at both sites. Bulbs were subsequently graded by size and quality according to U.S. Department of Agriculture grading standards (USDA 1995). Grades were determined by bulb diameter: cull (< 3.8 cm), small (3.8 to 5.7 cm), medium (5.7 to 7.6 cm), jumbo (7.6 to 10.2 cm), colossal (10.2 to 10.8 cm), and super colossal (> 10.8 cm). Bulbs in the medium to super colossal grades formed the U.S. no. 1 category.

**Shikimic Acid Accumulation.** A modified spectrophotometric method (Pline et al. 2001; Singh and Shaner 1998) as applied in Felix et al. (2011) was used to quantify shikimic acid accumulation in the onion leaf tissue. Ten plants were randomly selected from the two center rows of each plot at 7 DAT, and a single youngest leaf collected from each plant and pooled. Leaf samples were harvested in order from zero glyphosate to the highest dose. The leaf samples were immediately placed in a sealed plastic bag and transported to the laboratory in an iced cooler at 4 C. Samples from each site were packaged and transported to Pullman, WA for shikimic acid determination as described in Felix et al. (2011). Extraction techniques followed those of Pline et al. (2002), Singh and Shaner (1998), and Felix et al. 2011 with significant modifications. Subsamples of the onion leaves were placed in 1.5-ml centrifuge tubes and lyophilized. Subsample leaf weights were determined gravimetrically. The plant material was then ground in the microcentrifuge vial.

Table 1. Soil properties, planting date, herbicide spray timing, and air temperature for the simulated glyphosate drift study at Ontario, OR and Prosser, WA, 2011.

Soil and herbicide application timing			Air temperature <sup>c</sup>						
	Ontario, OR	Prosser, WA	Date	Prosser, WA			Ontario, OR		
				Max.	Min.	Mean	Max.	Min.	Mean
Soil type	Owyhee silt loam <sup>a</sup>	Warden sandy loam <sup>b</sup>		C					
pH	7.5	6.7	May 8	17.0	2.6	9.8	19.2	2.6	10.9
Organic matter (%)	1.25	1.07	May 16	20.1	5.2	12.7	21.6	6.5	14.1
Soil texture			May 24	20.0	5.5	12.7	20.6	7.0	13.8
Sand (%)	30.6	52	May 31	18.9	6.4	12.6	17.5	5.3	11.4
Silt (%)	55.4	39.2	June 6	22.1	8.7	15.4	21.5	8.5	15.0
Clay (%)	14	8.8	June 16	23.2	8.2	15.7	23.0	9.0	16.0
Planting date	April 7, 2011	April 15, 2011	June 24	24.8	9.3	17.0	26.8	10.1	18.4
Spraying timings			June 30	25.5	10.7	18.1	28.4	11.8	20.1
Onion at flag-leaf stage	May 11, 2011	May 17, 2011	July 8	28.9	10.6	19.7	32.3	13.5	22.9
Onion at two-leaf stage	May 24, 2011	May 31, 2011	July 16	26.1	11.0	18.6	30.5	12.8	21.6
Onion at four-leaf stage	June 10, 2011	June 17, 2011	July 24	27.1	10.8	19.0	30.9	12.4	21.7
Onion at six-leaf stage	June 28, 2011	July 7, 2011	July 31	29.3	12.4	20.8	33.5	13.2	23.3
			August 8	30.4	12.7	21.5	34.7	14.2	24.5
			August 16	28.3	9.8	19.1	32.5	12.2	22.3
			August 24	31.3	13.6	22.4	34.2	12.4	23.3
			August 31	30.9	14.1	22.5	34.8	13.5	24.1

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

<sup>b</sup> Warden sandy loam (Coarse-silty, mixed, superactive, mesic Xeric Haplocambids).

<sup>c</sup> Each value represents the average air temperature for the days preceding the date within each month. Abbreviations; Max, maximum temperature; min, minimum temperature; mean, average temperature.

After maceration, 0.25 ml of 0.4 M NaCO<sub>3</sub> was added and the extract was agitated and then centrifuged at 10,000 × *g* for 4 min. The extract was analyzed immediately. Two 20-μl aliquots of each sample were mixed in 0.5 ml of 1% wt/v periodic acid in separate 200-μl wells of a 96-well plate and allowed to oxidize. After 3 h, 0.5 ml of 1 N NaOH was added to the sample well and 0.5 ml of deionized water was added to the sample standard well. 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 milligram of dry weight of onion leaf on the basis of a standard curve (Felix et al. 2011; Pline et al. 2002; Singh and Shaner 1998). Standard curves were developed by using pure shikimic acid standards of known concentrations.

**Statistical Analysis.** The data were subjected to a normality test for compliance with the assumptions for statistical analysis. Nontransformed data were used in the final ANOVA because square-root-transformed data yielded similar ANOVA results. The data were subjected to ANOVA using PROC GLM procedure in SAS (SAS 2008). Type III statistics were used to test for significant differences ( $P \leq 0.05$ ) of sites, glyphosate dose, application timings, and their interactions for plant injury, height, and bulb yield variables. Data were pooled across sites or timings when no significant effects for site, timing, or site-by-timing interactions were detected. Pearson correlation coefficients ( $P < 0.00001$ ) were determined among onion injury, plant height, shikimic acid accumulation, and U.S. no. 1 onion yield. Pearson's correlations are utilized to measure the strength of the linear relationship between two variables

(Lassiter et al. 2007). Regression of onion plant injury ratings, height, shikimic acid accumulation, and yield over herbicide dose was achieved using a four-parameter log-logistic model as described by Seefeldt et al. (1995) and indicated below:

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

where *Y* is the response (e.g., percentage of onion 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*<sub>50</sub>]). Analysis of the dose–response curves and ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>25</sub> (the dose to inflict 5, 10, and 25% injury) values were determined using the open-source R language statistical package (R Development Core Team 2009), and the drc package in R (Ritz and Streibig 2005) as described by Knezevic et al. (2007). Comparison of means was performed with the use of Fisher's Protected LSD test at  $P \leq 0.05$ .

## Results and Discussion

**Plant Injury.** The data for foliar injury evaluations at 7 DAT were combined across sites because ANOVA indicated no significant difference between sites, but there was an interaction of glyphosate application timing with glyphosate dose (Figure 1). Onion foliar injury was characterized by chlorosis of the newest leaves that was visible as early as 3 DAT. The injury was also accompanied by “leaf tip burn” and general stunting of plants. Onion foliar injury at 7 DAT varied by application timing and was related directly to glyphosate dose. Onion injury at glyphosate  $\leq 25.8$  g ha<sup>-1</sup> ranged from 0 to 12%. The injury increased in severity with



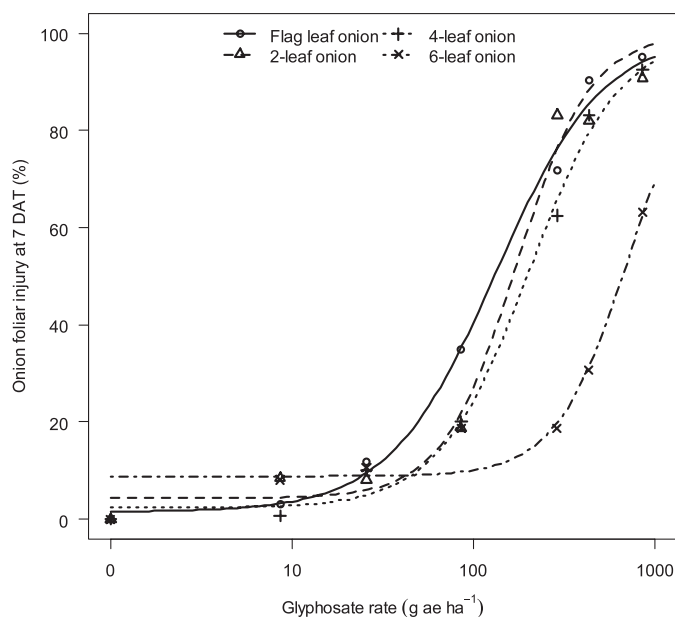


Figure 1. Onion foliar injury at 7 d after treatment in response to simulated glyphosate drift in field studies at Ontario, OR and Prosser, WA in 2011. Nonlinear regression lines were fit to combined site data using Equation 1. Parameter estimates for glyphosate doses required to impart 5, 10, and 25% foliar injury are presented in Table 2. Values in the  $x$ -axis are in log scale.

the increase in glyphosate dose and eventually resulted in the death of plants treated with glyphosate at  $860 \text{ g ha}^{-1}$  at flag-through four-leaf stages (Figure 1). Injury for plants sprayed at the six-leaf stage increased sharply at glyphosate rates  $\geq 290 \text{ g ha}^{-1}$  and peaked at 63% for the highest dose.

The calculated  $I_{50}$  at 7 DAT was 133.5, 173, 203.8, and  $728.4 \text{ g ha}^{-1}$  for plants sprayed at the flag-, two-, four-, and six-leaf stages, respectively (Table 2). The corresponding doses for  $ED_5$  to  $ED_{25}$  ranged from 18.2 to 63.4, 43.3 to 103.2, 37.4 to 108.3, and 186.7 to  $438.3 \text{ g ha}^{-1}$ , 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. Even though glyphosate has been reported to act slowly in treated plants (Stoller et al. 1975), the injury on onion was evident as early as 3 DAT. It is unclear why onion responded so fast to glyphosate. Probably the cooler conditions at application may have reduced onion leaf cuticle formation and in turn increased glyphosate uptake by the young plants. Onion has been reported to be susceptible to POST herbicide injury before the two-leaf stage because the plant has yet to form its protective leaf cuticle (Akey and Souza Machado 1985; Ashton and Monaco 1991).

No significant difference between sites ( $Pr > F, 0.117$ ) was indicated by ANOVA for the onion foliar injury at 21 DAT, but there was a glyphosate rate-by-application timing interaction (Figure 2). Foliar injury severity increased with the increase in glyphosate dose. The injury increased when glyphosate was applied at  $25.8 \text{ g ha}^{-1}$  or greater for plants that were at flag- and four-leaf stages, and ranged from 24 to 99%

among doses. Injury to plants treated at the two- and six-leaf stages increased when glyphosate was applied at  $\geq 86 \text{ g ha}^{-1}$ , and ranged from 24 to 97% and 28 to 80%, respectively. The  $I_{50}$  glyphosate dose at 21 DAT was lowest when onion was treated at four- and flag-leaf stages and was estimated to be 76.8 and  $81 \text{ g ha}^{-1}$ , respectively (Table 2). These doses are in the range expected from a typical downwind herbicide drift, which has been estimated to range from 1 to 8% of the applied dose (Bode 1987; Maybank et al. 1978) depending on prevailing wind speed. The corresponding  $I_{50}$  for onions sprayed at two- and six-leaf stages was 1.72 and 2.45 times greater compared with plants sprayed at the flag-leaf stage, respectively. The  $ED_5$ ,  $ED_{10}$ , and  $ED_{25}$  (glyphosate dose to result in 5, 10, and 25% foliar injury) at 21 DAT followed the similar trend as the  $I_{50}$  doses. The  $I_{50}$  dose pattern at 21 DAT was four-leaf < flag-leaf < six-leaf < two-leaf. These results indicated that onion sensitivity to glyphosate was greater at the four- and flag-leaf stages compared with the two- and six-leaf stages. Increased plant injury in relation to glyphosate dose in simulated drift studies has been reported in other crops, including potato (Felix et al. 2011), sorghum (Al-Khatib et al. 2003), tobacco (Burke et al. (2005), rice (*Oryza sativa* L.) (Ellis et al. 2003; Koger et al. 2005), and peanuts (*Arachis hypogea* L.) (Lassiter et al. 2007). It is unclear why onion at the four-leaf stage was more sensitive to glyphosate compared with plants at the two-leaf stage. It is likely that variation in air temperature at the time of glyphosate application could have influenced the results.

The  $I_{50}$  and the shape of the dose–response curve can change with time after treatment (Burke et al. 2005). There was a slight shift of the four-leaf regression line toward the left, suggesting enhanced injury at 21 DAT for plants sprayed with low doses of glyphosate. These results are consistent with slow action of glyphosate in plants (Stoller et al. 1975).

**Plant Height.** There was site-by-glyphosate application timing and site-by-glyphosate dose interactions for onion plant height at 35 DAT, necessitating the data to be presented separately for each site (Figure 3). The increase in glyphosate dose resulted in reduced plant height regardless of the application timing at Ontario, OR (Figure 3A). Herbicides that inhibit amino acid synthesis often reduce leaf size and internode length (Eberlein et al. 1997) and hence reduce the plant height. Variation in plant height among glyphosate application timings was expected because the herbicide was applied to onion at different growth stages. There was a precipitous drop in plant height when glyphosate rates  $\geq 8.6 \text{ g ha}^{-1}$  were applied at the four-leaf stage at Ontario, OR. Height of plants treated at the two-leaf stage was reduced when glyphosate was applied at  $\geq 25.6 \text{ g ha}^{-1}$ , compared with  $\geq 86 \text{ g ha}^{-1}$  for plants sprayed at the flag-leaf stage. There was a gradual decline in plant height for plants treated at the six-leaf stage. These results indicated that plant growth stage played a role in the onion response to glyphosate doses, and the susceptibility was much enhanced at the four-leaf stage. Greater foliar injury and larger reductions in plant height were observed at 7 and 21 DAT for onion plants treated at the four-leaf stage. Development of onion bulb is controlled by day length and generally the diameter begins to enlarge at about the four-leaf stage (as depicted in Brewster

Table 2. Regression parameter estimates and glyphosate dose (g ae ha<sup>-1</sup>) to impart 5, 10, and 25% bulb onion plant injury (ED<sub>5,10,25</sub> [±SE]) based on ratings at 7, 21, and plant height at 35 d after treatment and U.S. no. 1 onion yield.<sup>a</sup>

Variable	Timing	Regression parameters (± SE)						
		<i>B</i>	<i>C</i>	<i>D</i>	<i>I</i> <sub>50</sub>	ED <sub>5</sub> (± SE)	ED <sub>10</sub> (± SE)	ED <sub>25</sub> (± SE)
Injury 7 DAT (combined)	Flag-leaf stage	-1.5 (0.17)	1.53 (2.96)	100	133.5 (14.8)	18.2 (5.5)	30.1 (7.4)	63.4 (10.8)
	Two-leaf stage	-2.1 (0.29)	4.35 (2.4)	100	173.0 (14.5)	43.3 (9.9)	61.6 (11.4)	103.2 (12.9)
	Four-leaf stage	-1.7 (0.23)	2.36 (2.7)	100	203.8 (20.0)	37.4 (11.2)	57.5 (14.0)	108.3 (17.8)
	Six-leaf stage	-2.2 (0.41)	8.90 (2.1)	100	728.4 (52.6)	186.7 (46.6)	263.7 (49.6)	438.3 (46.3)
Injury 21DAT (Combined)	Flag-leaf stage	-1.1 (0.23)	0.84 (4.3)	108.1 (8.13)	81.0 (17.5)	6.0 (2.9)	11.6 (4.2)	30.6 (6.8)
	Two-leaf stage	-2.8 (0.66)	5.3 (2.8)	95.9 (4.85)	139.6 (19.5)	47.9 (10.4)	62.9 (10.5)	93.7 (11.3)
	Four-leaf stage	-1.0 (0.21)	-0.2 (4.3)	106.1 (8.64)	76.8 (19.0)	4.6 (2.3)	9.4 (3.5)	26.8 (6.3)
	Six-leaf stage	-1.2 (0.40)	3.3 (3.9)	93.1 (16.79)	198.8 (75.4)	17.3 (10.5)	32.2 (13.8)	80.0 (21.2)
Shikimic acid (Ontario)	Flag-leaf stage	-3.3 (3.19)	12.5 (20.6)	219.3 (53.6)	282.6 (81.5)	54.4 (59.2)	80.2 (64.5)	141.8 (71.5)
	Two-leaf stage	-1.9 (1.64)	5.6 (24.1)	254.9 (95.1)	250.7 (144.5)	8.2 (4.2)	17.2 (6.5)	50.8 (16.2)
	Four-leaf stage	-1.0 (0.26)	-29.3 (33.2)	791.1 (138.2)	150.6 (69.6)	116.7 (98.6)	146.1 (94.8)	203.2 (79.3)
	Six-leaf stage	-1.0 (0.42)	27.8 (26.6)	988.9 (122.4)	2496.6 (286.2)	270.7 (114.8)	581.2 (255.1)	1787.3 (857.1)
Shikimic acid (Prosser)	Flag-leaf stage	-0.8 (0.44)	10.6 (18.9)	568. (73.7)	247.2 (41.7)	13.6 (8.5)	28.4 (13.9)	83.8 (25.0)
	Two-leaf stage	-1.1 (0.18)	32.3 (12.9)	1,628.0 (63.1)	896.8 (365.5)	1.6 (4.0)	12.0 (19.2)	233.5 (112.7)
	Four-leaf stage	-5.6 (12.65)	5.5 (10.7)	276.4 (306.8)	596.9 (56.8)	336.7 (55.1)	394.1 (52.7)	496.8 (47.8)
	Six-leaf stage	-5.1 (12.30)	25.6 (10.9)	1,380.6 (162.9)	1,341.6 (428.9)	270.2 (115.9)	364.2 (112.6)	564.8 (84.1)
Plant height (Ontario)	Flag-leaf stage	2.0 (0.82)	3.74 (3.0)	22.3 (1.22)	182.5 (61.8)	42.0 (25.6)	61.0 (28.3)	105.5 (35.8)
	Two-leaf stage	0.7 (0.36)	-8.9 (30.7)	36.5 (2.11)	430.6 (866.6)	5.8 (4.7)	17.3 (12.8)	86.4 (108.4)
	Four-leaf stage	0.7 (0.17)	11.8 (5.1)	56.0 (1.99)	45.8 (20.3)	0.7 (0.6)	2.1 (1.1)	9.8 (3.2)
	Six-leaf stage	0.5 (0.30)	36.0 (18.6)	66.2 (2.18)	267.5 (670.2)	0.8 (1.4)	3.5 (4.4)	30.7 (46.0)
Plant height (Prosser)	Flag-leaf stage	0.7 (0.23)	-0.04 (8.6)	33.5 (2.3)	129.4 (119.9)	1.5 (1.7)	4.5 (4.1)	24.2 (16.9)
	Two-leaf stage	1.8 (0.63)	-0.03 (4.0)	28.8 (1.5)	169.4 (47.2)	31.8 (17.2)	48.6 (20.0)	90.8 (24.6)
	Four-leaf stage	1.2 (0.40)	-11.3 (14.6)	44.3 (1.8)	364.0 (173.4)	31.4 (15.2)	58.4 (19.2)	145.9 (40.3)
	Six-leaf stage	0.8 (0.35)	18.6 (11.1)	51.4 (2.2)	159.6 (160.8)	3.9 (4.0)	9.9 (7.3)	39.8 (25.0)
Yield (Ontario)	Flag-leaf stage	4.8 (1.74)	5.5 (8.2)	94.0 (3.6)	335.9 (27.0)	181.6 (39.0)	212.3 (34.7)	267.0 (26.2)
	Two-leaf stage	1.8 (0.67)	-9.1 (17.3)	102.6 (4.6)	265.4 (59.1)	53.2 (27.8)	79.9 (31.1)	145.7 (33.4)
	Four-leaf stage	1.0 (0.27)	-8.4 (9.5)	99.8 (7.0)	39.9 (13.3)	2.0 (1.5)	4.3 (2.5)	13.1 (4.7)
	Six-leaf stage	2.7 (0.94)	0.6 (7.7)	90.0 (4.3)	139.9 (31.3)	46.1 (15.4)	61.1 (15.8)	92.4 (17.7)
Yield (Prosser)	Combined	0.9 (0.14)	0	95.9 (5.8)	47.3 (12.1)	2.1 (1.4)	4.6 (2.5)	14.8 (5.8)

<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, 10, 25</sub>, the glyphosate dose needed to cause 5, 10, and 25% foliar injury or plant height reduction or U.S. no. 1 yield reduction compared with the untreated control; DAT, days after treatment. Glyphosate sprays included ammonium sulfate at 2.5% v/v.

2008). This physiological stage marks rapid extension in leaf number and size and could have rendered the plants more susceptible to a translocated herbicide like glyphosate. The *I*<sub>50</sub> dose at Ontario was 45.8 < 182.5 < 267.5 < 430.6 g ha<sup>-1</sup> for plants sprayed at four-, flag-, six-, and two-leaf stage, respectively (Table 2). The ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>25</sub> for plant height at Ontario were lowest at the four- and six-leaf stages (0.7, 2.1, 9.8 and 0.8, 3.5, and 30.7 g ha<sup>-1</sup>, respectively).

Onion plant height at Prosser varied by glyphosate application timing and doses, and is presented in Figure 3B. Glyphosate rates ≤ 25.6 g ha<sup>-1</sup> elicited minimal reduction in plant height compared with the nontreated control. Plant height for the nontreated control at 35 DAT was 34, 30, 45, and 51 cm for plants treated at the flag-, two-, four-, and six-leaf stage, respectively. The corresponding plant height reduction from glyphosate ≥ 86 g ha<sup>-1</sup> was 43 to 80%, 27 to 96%, 22 to 91%, and 22 to 51% relative to the nontreated control, respectively. The *I*<sub>50</sub> dose for plant height at Prosser, WA ranged from 169.4 g ha<sup>-1</sup> to 364 g ha<sup>-1</sup> among herbicide application timings (Table 2). The ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>25</sub> for plant height were lowest for plants sprayed at flag- and six-leaf stages. Differences in weather between sites may have contributed to the observed results. Because of cold weather, onion was planted later at Prosser and glyphosate application timings were about 7 d after those at Ontario. Importantly, air

temperature starting late June was 1 to 3 C higher in Ontario (Table 1). Norsworthy et al. (2007) reported reduced plant height when glyphosate was applied at 280 or 560 g ha<sup>-1</sup> to green onion. In the same study, they noted yearly variability in crop response to glyphosate over the 2-yr period and suggested that air temperature variations may have been the cause for the differential response.

There was a strong negative correlation between onion plant injury at 7 and 21 DAT and U.S. no. 1 dry bulb onion yield as well as a strong positive correlation between plant height and U.S. no. 1 dry bulb onion yield. Correlations for injury at 7 DAT and yield were -0.72 < *r* < -0.91 and -0.69 < *r* < -0.76 at Ontario and Prosser, respectively. The correlations for onion injury at 21 DAT and yield were -0.83 < *r* < -0.98 at Ontario and -0.75 < *r* < -0.81 at Prosser. These results indicated that the increased injury at 7 and 21 DAT was followed by a concomitant reduction in onion yield. Plant height and onion yield were also strongly correlated (0.84 < *r* < 0.97 and 0.75 < *r* < 0.83) at Ontario and Prosser, respectively. These results indicated that decrease in plant height in response to glyphosate application was accompanied by a decrease in U.S. no. 1 onion yield at each site. The results corroborate the findings by Norsworthy et al. (2007) that glyphosate reduces plant height in onion and is possibly influenced by the prevailing air temperature.

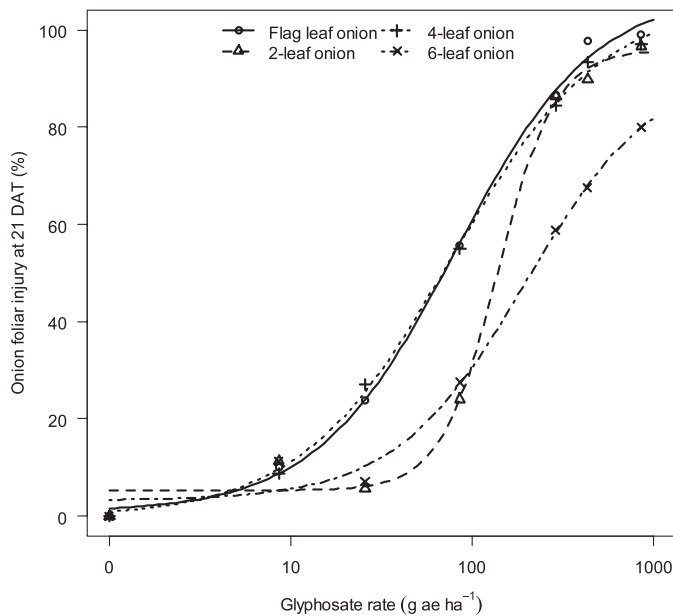


Figure 2. Onion foliar injury at 21 d after treatment with various glyphosate doses to onion at different onion growth stages in field studies conducted at Ontario, OR and Prosser, WA in 2011. Nonlinear regression lines were fit using Equation 1. Regression parameter estimates for glyphosate doses required to produce 5, 10, and 25% foliar injury are presented in Table 2. Values in the  $x$ -axis are in log scale.

**Shikimic Acid Accumulation.** There was a significant site-by-treatment interaction for shikimic acid accumulation; therefore, the data are presented separately for each site. Shikimic acid accumulation increased as the rate of glyphosate increased (Figure 4). There was a strong relationship between plant injury at 7 and 21 DAT and shikimic acid

accumulation, plant height at 35 DAT and shikimic acid accumulation, and onion yield and shikimic acid accumulation at both sites (Figures 1–5). In samples taken 7 DAT, shikimic acid accumulation increased with the increase in glyphosate dose, although the magnitude of the increase depended on the application timing (Figure 4). At Ontario, shikimic acid accumulation was highest (5.1 to 684 mg g<sup>-1</sup>) when glyphosate 8.6 to 860 g ha<sup>-1</sup> was applied at the four-leaf stage (Figure 4A). The corresponding shikimic acid accumulation for plants treated at the flag-leaf stage, which had the lowest amount, was 0 to 218 mg g<sup>-1</sup>. Samples taken 7 DAT at Prosser indicated that shikimic acid accumulation was highest at flag- and four-leaf stage (11.8 to 256.4 mg g<sup>-1</sup> and 8.3 to 245.8 mg g<sup>-1</sup>), and lowest (8.6 to 128 mg g<sup>-1</sup>) for plants sprayed at the two-leaf stage (Figure 4B). The differences for shikimic acid accumulation at Ontario and Prosser could be attributed to differences in prevailing temperatures. Extended low temperatures at Prosser (Table 1) may have slowed plant metabolism and contributed to slower shikimic acid accumulation. Lower shikimic acid accumulation could also be attributed to reduced or delayed glyphosate transport—because glyphosate is transported in the phloem of actively growing plants (Bromilow and Chamberlain 2000).

There was a positive correlation between shikimic acid accumulation and foliar injury at 7 DAT and 21 DAT at both sites. Correlation coefficients for shikimic acid accumulation at 7 DAT were  $0.81 < r < 0.95$  and  $0.51 < r < 0.86$  at Ontario and Prosser, respectively. The corresponding accumulation at 21 DAT was  $0.82 < r < 0.90$  and  $0.50 < r < 0.77$  at Ontario and Prosser, respectively. These results indicated that both shikimic acid and foliar injury increased as glyphosate dose increased. Similar results were reported in peanut by Lassiter et al. (2007) and in tobacco by Burke et al. (2005). There was a negative correlation for shikimic acid accumulation

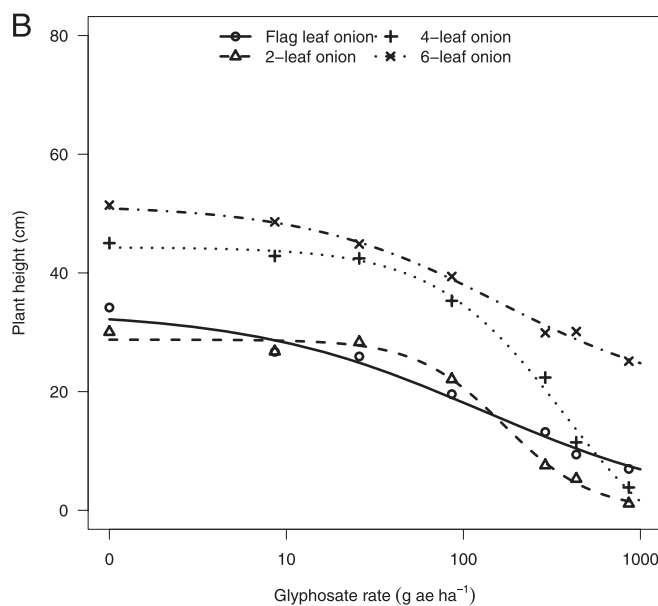
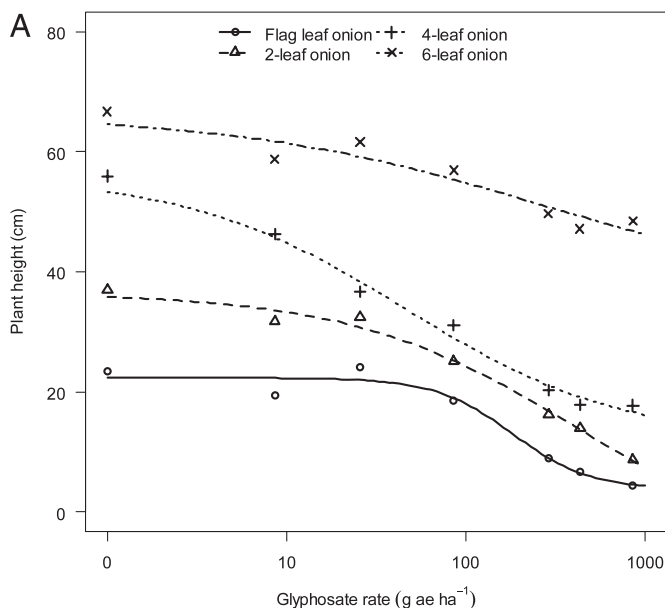


Figure 3. Onion plant height at 35 d after treatment with variable glyphosate doses applied to onion plants at different growth stages in field studies at Ontario, OR (A) and Prosser, WA (B) in 2011. Nonlinear regression parameter estimates for glyphosate doses required to produce 5, 10, and 90% foliar injury are presented in Table 2. Values in the  $x$ -axis are in log scale.

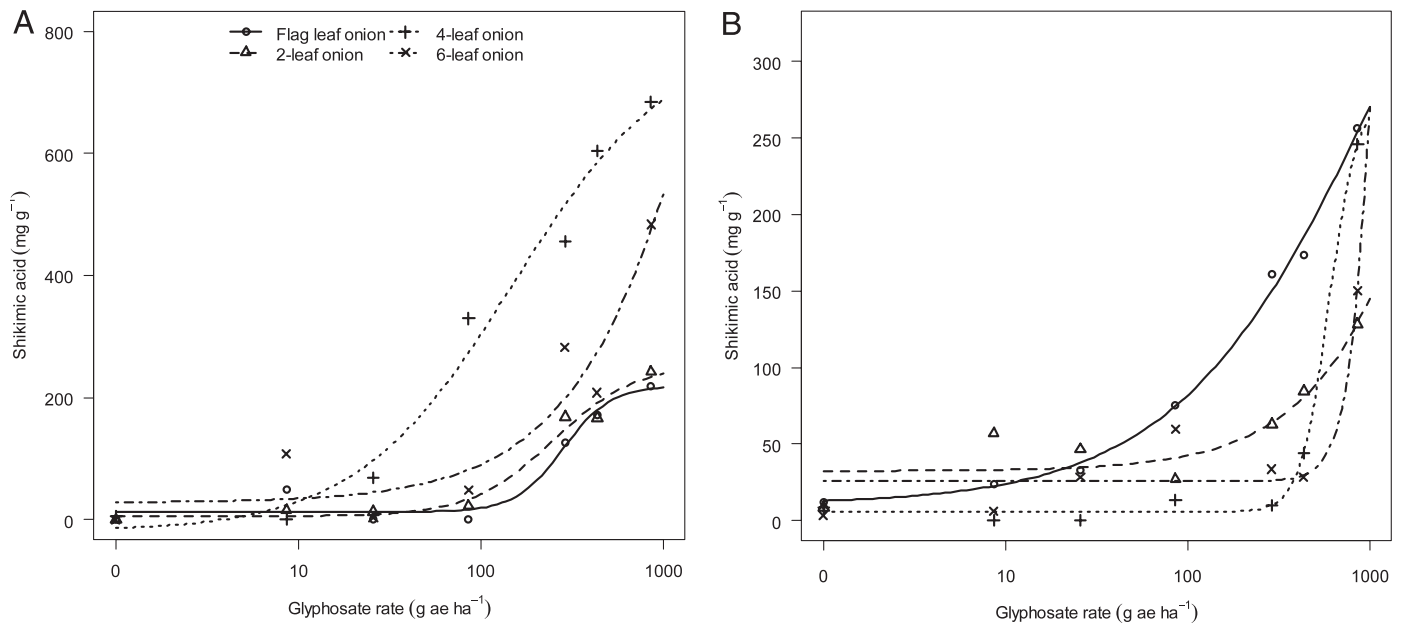


Figure 4. Shikimic acid accumulation for onion leaf samples taken 7 d after treatment with incremental glyphosate doses to onion plants at different growth stages at Ontario, OR (A) and Prosser, WA (B) in 2011.

and onion yield ( $-0.85 < r < -0.89$  and  $-0.40 < r < -0.57$  at Ontario and Prosser, respectively), indicating that onion yield decreased as shikimic acid accumulation increased. These results indicated that sampling for shikimic acid at 7 DAT could accurately predict onion yield loss, and possibly be used as a tool by farm managers and agricultural consultants to advise growers on the action to be taken in an event of early-season glyphosate drift on direct-seeded dry bulb onion.

**Onion Yield.** There was a significant site-by-treatment interaction for onion yield, and thus, the results are presented separately by site (Table 3). Bulb yield at Prosser varied among glyphosate doses, but was similar across application timings within each grade. The level of culled bulbs ( $< 2.5$  cm) increased as glyphosate dose increased from  $290 \text{ g ha}^{-1}$  or greater at both sites. The highest culled yield was observed for plants sprayed at the six-leaf stage at Ontario. Yield for the

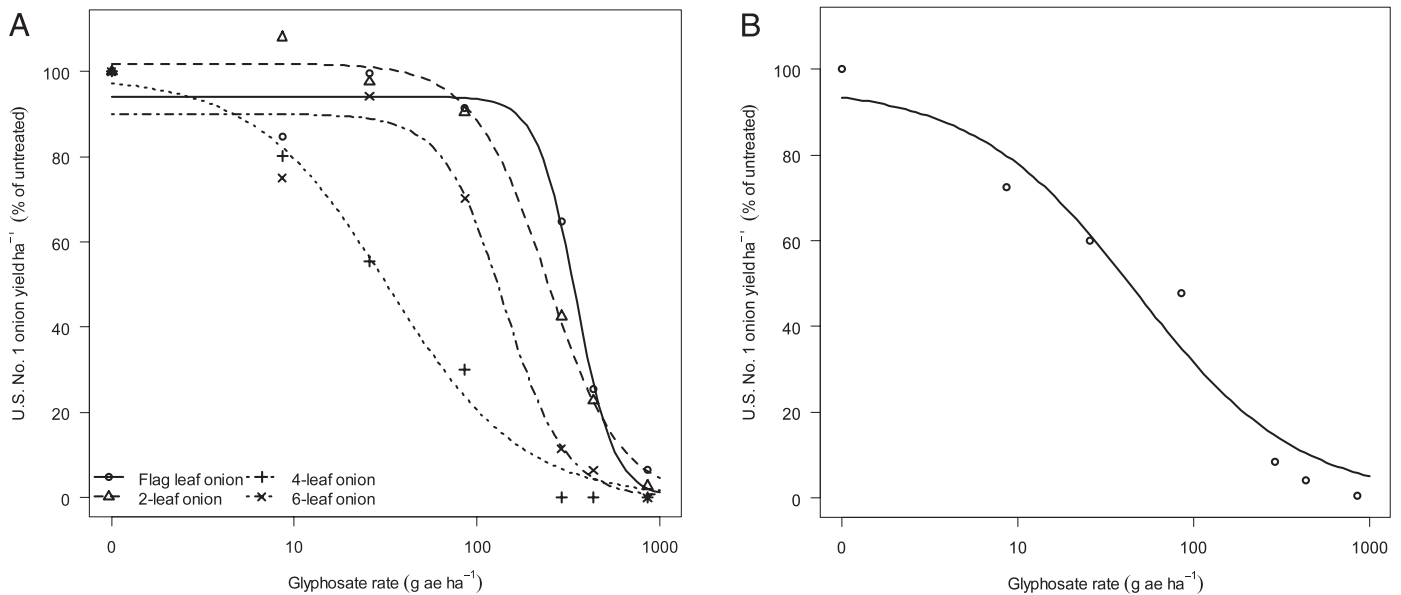


Figure 5. Relative U.S. no. 1 dry bulb onion yield at Ontario, OR (A) and Prosser, WA (B) in response to simulated glyphosate drift at different onion growth stages. The average bulb yield for the untreated controls at Ontario, OR was  $120 \text{ MT ha}^{-1}$  and  $38.3 \text{ MT ha}^{-1}$  at Prosser, WA. Nonlinear regression lines were fit to combined site data using Equation 1. Parameter estimates for glyphosate doses required to impart 5, 10, and 25% foliar injury are presented in Table 2. Values in the x-axis are in log scale.



Table 3. Effect of simulated glyphosate drift on cull, small, medium, jumbo, and colossal plus super colossal onion grade yield<sup>a</sup> at Ontario, OR and Prosser, WA, 2011.

		Onion yield at Ontario, OR																			
Herbicide	Rate	Cull				Small				Medium				Jumbo				Colossal + super colossal			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Untreated	0	0.0				0.4				6.2				73.8				40.0			
Glyphosate	8.6	0.0	0.0	0.0	1.9	1.1	0.2	1.4	1.9	3.9	3.0	7.7	6.4	54.6	87.6	74.1	66.5	35.6	35.1	17.7	20.1
	25.8	0.0	0.0	0.0	0.0	0.4	0.2	2.9	0.3	3.3	3.8	15.6	2.6	72.6	79.0	48.3	85.4	37.8	31.6	5.8	29.5
	86	0.0	0.0	0.0	0.0	0.8	0.5	6.4	1.2	5.0	3.3	18.7	10.5	70.5	77.6	18.5	71.2	29.7	24.8	0.1	6.0
	290	0.1	0.2	0.2	3.4	1.3	4.6	0.6	9.5	6.2	10.8	0.0	12.5	47.2	36.2	0.1	1.6	22.6	2.3	0.0	0.0
	434	0.1	0.1	0.2	3.6	2.0	4.2	0.0	7.1	6.1	9.7	0.1	7.4	20.4	14.3	0.0	0.7	3.5	1.9	0.0	0.0
	860	0.0	0.0	0.0	1.6	1.5	1.5	0.0	0.6	2.2	1.7	0.0	0.0	5.2	1.5	0.0	0.0	0.5	0.0	0.0	0.0
LSD <sub>0.05</sub>		NS				2.0				4.0				22.5				19.4			
		Onion yield at Prosser, WA																			
Untreated	0	0.0				4.5				17.8				20.5				3.6			
Glyphosate	8.6	0.1	0.1	0.0	0.0	5.0	3.5	4.8	5.5	10.7	14.5	17.6	14.4	5.5	11.6	12.3	9.0	4.8	2.5	0.0	0.4
	25.8	0.0	0.0	0.0	0.1	6.4	4.7	4.6	7.2	12.7	18.3	13.6	14.4	4.4	11.0	10.3	6.9	0.0	0.0	0.5	0.0
	86	0.2	0.0	0.0	0.0	2.7	6.2	5.6	6.7	6.1	15.9	9.3	14.4	7.8	5.2	10.7	3.9	0.9	0.0	0.5	0.0
	290	0.0	0.0	0.3	0.1	1.8	3.9	7.4	8.8	1.7	3.3	0.9	1.3	1.2	2.3	0.0	0.0	0.0	0.3	0.0	0.0
	434	0.0	0.2	0.2	0.0	0.7	4.1	2.9	10.7	1.0	3.4	0.5	0.3	0.5	0.8	0.0	0.0	0.0	0.0	0.0	0.0
	860	0.0	0.0	0.0	0.5	0.3	0.3	0.0	3.9	0.4	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD <sub>0.05</sub>		NS				2.9				5.5				6.5				NS			

<sup>a</sup> Onion grades are based on bulb diameter: cull (< 3.8 cm), small (3.8 to 5.7 cm), medium (5.7 to 7.6 cm), jumbo (7.6 to 10.2 cm), colossal (10.2 to 10.8 cm), plus super colossal (> 10.8 cm). Abbreviations; A, glyphosate applied when onion was at flag-leaf stage; B, when onion was at two-leaf stage; C, when onion was at four-leaf stage; and D, when onion was at six-leaf stage.

small bulb (2.5 to 5.7 cm) category varied greatly across glyphosate doses and application timings (0 to 9,500 kg ha<sup>-1</sup> at Ontario and 0 to 7,400 kg ha<sup>-1</sup> at Prosser). At Ontario, medium sized bulb yield was similar across glyphosate doses when glyphosate was applied at the flag-leaf stage (2,200 to 6,200 kg ha<sup>-1</sup>), but varied greatly (0 to 18,700 kg ha<sup>-1</sup>) for plants treated at the two- to six-leaf stages. At Prosser, medium onion yield generally declined when glyphosate was applied 290 g ha<sup>-1</sup> or greater. Yield for jumbo, colossal, and super colossal declined as glyphosate dose increased regardless of the application timing at both sites.

The U.S. no. 1 yield category, which was composed of medium, jumbo, colossal, and super colossal bulb grades is presented by site in Figure 5. The relative bulb yield (percentage of the nontreated) at Ontario for plants sprayed with glyphosate at different growth stages was directly related to the level of foliar injury observed earlier in the season (Figures 1 and 2) and plant height at 35 DAT (Figure 3). There was an inverse relationship between plant injury and onion yield (Figures 1, 2, and 5A). As foliar injury increased at Ontario in response to increased glyphosate doses at different application timings, the plant height gradually decreased and the bulb yield decreased accordingly (Figure 5A). The U.S. no. 1 bulb yield for the nontreated controls at Ontario averaged 120,000 kg ha<sup>-1</sup> for plants treated at flag-, two-, four-, and six-leaf stages. Onion yield was reduced 15 to 93% when glyphosate 8.6 to 860 g ha<sup>-1</sup> was applied at the flag-leaf stage. Respective yield for plants sprayed with glyphosate at the two-leaf stage was reduced 2 to 97%. Bulb yield was reduced 20 to 70% with glyphosate 8.6 to 86 g ha<sup>-1</sup> applied at the four-leaf stage. There were no U.S. no. 1 bulbs produced when glyphosate was applied ≥ 290 g ha<sup>-1</sup> at the four-leaf stage. The I<sub>50</sub> doses for onion yield at Ontario when glyphosate was applied at the four-, six-, two-, and flag-leaf

stage were estimated to be 39.9, 139.9, 265.4, and 335.9 g ha<sup>-1</sup>, respectively (Table 2). The glyphosate doses to reduce onion yield 5, 10, and 25% were 2, 4.3, and 13.1 g ha<sup>-1</sup> for plants treated at the four-leaf stage. Glyphosate 46.1 to 145.7 g ha<sup>-1</sup> was needed to elicit the same yield reduction to plants treated at the two- and six-leaf stages. The estimated glyphosate dose to reduce yield 5 to 25% at Ontario was 181.6 to 267 g ha<sup>-1</sup> when plants were sprayed at the flag-leaf stage.

Several reasons may explain the lower yield response for plants sprayed at the flag-leaf stage. Some plants may have recovered from injury because seed reserves were still being used to support the newly emerged seedlings. The possibility exists that affected plants may have developed new shoots that grew to produce normal bulbs. Also, other seedlings may have not emerged or not fully emerged when glyphosate was applied and may not have received enough herbicide doses to elicit injury. 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. Onion yield for plants sprayed at the flag-leaf stage at Ontario was relatively greater than other timings at doses ≤ 290 g ha<sup>-1</sup>.

The ANOVA indicated no significant application timing effects for the U.S. no. 1 onion yield at Prosser and the data were subsequently pooled and analyzed (Figure 5B). Bulb yield was reduced 27, 40, and 51% relative to the nontreated control (41,900 kg ha<sup>-1</sup>) when glyphosate was applied at 8.6, 25.6, and 86 g ha<sup>-1</sup>, respectively. Application of glyphosate at ≥ 290 g ha<sup>-1</sup> reduced the U.S. no. 1 yield 91 to 99% relative to the nontreated control. These results are similar to those at Ontario for glyphosate ≥ 290 g ha<sup>-1</sup> doses. The I<sub>50</sub> dose at Prosser was estimated to be 47.3 g ha<sup>-1</sup>, whereas the ED<sub>5</sub>, ED<sub>10</sub>, and ED<sub>25</sub> doses were 2.1, 4.6, and 14.8 g ha<sup>-1</sup>,



respectively. The reduced U.S. no.1 onion yield for plants sprayed with glyphosate at  $\geq 290 \text{ g ha}^{-1}$  at the flag-, two-, and four-leaf stages was attributed to foliar injury and reduced plant height. Compared with Ontario, the weather was relatively cool at Prosser throughout the growing season (Table 1). Cool conditions likely affected plant growth, reduced plant height as glyphosate doses increased, and culminated in reduced onion yields.

Simulated glyphosate drift also increased the percentage of bulbs  $\leq 5.7 \text{ cm}$  diam at both sites, which are considered unmarketable (Table 3). There also was delayed maturity characterized by plants with “thick necks” that were still green at the time of harvest. In addition, there was an increase in the number of rotten bulbs at the two highest glyphosate doses. As was the case at Ontario, the relationship between onion injury and U.S. no. 1 yield at Prosser was strongly negatively correlated ( $-0.75 < r < -0.83$ ). The yield results at both sites further indicated that onion is sensitive to glyphosate even at doses as low as 1% of the recommended dose ( $860 \text{ g ha}^{-1}$ ). Downwind drift deposits from nonshielded sprayers can be less than 1% to as much as 8% of the target dose (Bode 1987; Maybank et al. 1978) depending on prevailing wind. Norsworthy et al. (2007) reported excessive injury, ranging from 46 to 94% when glyphosate at  $560 \text{ g ha}^{-1}$  was applied to green onion. In that study, glyphosate at  $280 \text{ g ha}^{-1}$  caused 8 and 91% injury between years. They proposed that greater injury may have been a result of smaller onion size at the time of application.

It has been reported that in addition to the deleterious effects on potato tuber yield and quality, glyphosate drift also has a potential to negatively affect sprouting of daughter tubers derived from seed tuber production fields (Smid and Hiller 1981). It is unclear what would be the impact of glyphosate on onion bulbs grown for seed production. Also, many onions are stored for months following harvest and the effect of glyphosate drift on the storability of onion is important and will be addressed in a subsequent manuscript.

In summary, the results indicated greater injury to onion plants as glyphosate dose increased from  $8.6$  to  $860 \text{ g ha}^{-1}$  depending on the application timing. Glyphosate application at the four-leaf stage resulted in the largest reduction in U.S. no. 1 onion yield at Ontario, OR. Most growers would be able to identify injury on onion from a low glyphosate dose typical of accidental drift because of visible symptoms including chlorosis of newly emerged leaves and the reduction in plant height. In typical drift cases there is a concentration gradient downwind, with higher amounts deposited along the point of field entry or field margin. Growers or farm managers could compare visual injury following the prevailing wind direction at the time of application of the suspected drift. Because of the high production expense and the variability in onion market, most growers would be better advised to destroy the crop when significant injury symptoms result from glyphosate drift. Extreme diligence should be exercised when spraying glyphosate on field edges or glyphosate-tolerant crops in fields bordering onion. These results indicated that sampling for shikimic acid at 7 DAT could accurately predict onion

yield loss, and possibly be used as a tool by farm managers and agricultural consultants to both confirm glyphosate drift and advise growers on the action to be taken in an event of early-season glyphosate drift.

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