

Biological responses to glyphosate drift from aerial application in non-glyphosate-resistant corn

Krishna N Reddy,^a Wei Ding,^{a,b*} Robert M Zablotowicz,^a Steven J Thomson,^a Yanbo Huang^a and L Jason Krutz^a

Abstract

BACKGROUND: Glyphosate drift from aerial application onto susceptible crops is inevitable, yet the biological responses to glyphosate drift in crops are not well characterized. The objectives of this research were to determine the effects of glyphosate drift from a single aerial application (18.3 m swath, 866 g AE ha⁻¹) on corn injury, chlorophyll content, shikimate level, plant height and shoot dry weight in non-glyphosate-resistant (non-GR) corn.

RESULTS: One week after application (WAA), corn was killed at 3 m from the edge of the spray swath, with injury decreasing to 18% at 35.4 m downwind. Chlorophyll content decreased from 78% at 6 m to 22% at 15.8 m, and it was unaffected beyond 25.6 m at 1 WAA. Shikimate accumulation in corn decreased from 349% at 0 m to 93% at 15.8 m, and shikimate levels were unaffected beyond 25.6 m downwind. Plant height and shoot dry weight decreased gradually with increasing distance. At a distance of 35.4 m, corn height was reduced by 14% and shoot dry weight by 10% at 3 WAA.

CONCLUSIONS: Corn injury and other biological responses point to the same conclusion, that is, injury from glyphosate aerial drift is highest at the edge of the spray swath and decreases gradually with distance. The LD₅₀ (the lethal distance that drift must travel to cause a 50% reduction in biological response) ranged from 12 to 26 m among the biological parameters when wind speed was 11.2 km h⁻¹ and using a complement of CP-09 spray nozzles on spray aircraft.

Published 2010 by John Wiley & Sons, Ltd.

Keywords: aerial application; drift injury; glyphosate; off-target; shikimate; spray deposition

1 INTRODUCTION

The widespread adoption of glyphosate-resistant (GR) crops in the United States has led to an unprecedented increase in glyphosate usage in recent years. Glyphosate is the most commonly applied herbicide either alone or with other herbicides to manage a broad spectrum of weeds. Glyphosate is applied preplant to kill existing vegetation before planting spring-seeded crops, and post-emergence in GR crops. Glyphosate is applied multiple times in a year using either ground or aerial equipment. Pesticide drift, the physical movement of a pesticide particle onto an off-target, can occur when applied under weather conditions that promote drift. In virtually all pesticide applications, a small fraction of the pesticide drifts downwind and can be deposited on off-target surfaces. Glyphosate drift, however, is particularly important because it is a non-selective herbicide and highly active on sensitive plant species at low doses.

Aerial applications are often employed because of convenience and timeliness for effective weed control. Aerial application is the only method of choice for farmers during extended rainy periods and wet soil conditions, and for those who have limited farm equipment for timely glyphosate application. Drift continues to be a major problem in pesticide applications. A number of factors influence spray drift, including droplet size, release height, weather

conditions, topography, application equipment and methods and decisions by the applicator.

When aerial pesticide drift occurs, there is a rapid decline in surface deposition with increasing downwind distance from the target site. Bird *et al.*¹ analyzed the results of 72 new spray drift trials applied to a field of mowed grass and 45 trials previously reported in the literature. The authors concluded that, in general, pesticide deposits decreased from 5% of the nominal application rate at 30 m downwind to 0.5% at 150 m downwind during low-flight applications. Wind speeds over the 72 spray drift trials ranged from 4.7 to 22.7 km h⁻¹. In field crops, aerial glyphosate applications resulted in downwind drift of 65% at 10 m, 27% at 20 m, 6% at 40 m and 0.1% at 320 m, regardless of formulation.² Wind speeds in these four glyphosate formulation drift trials ranged from 12.1 to 17.4 km h⁻¹. Similarly, in forestry aerial application, glyphosate

* Correspondence to: Wei Ding, USDA-Agricultural Research Service, Crop Production Systems Research Unit, 141 Experiment Station Road, Stoneville, Mississippi 38776, USA. E-mail: Wei.Ding@ars.usda.gov

a USDA-Agricultural Research Service, Crop Production Systems Research Unit, Stoneville, Mississippi, USA

b Northeast Agricultural University, Agronomy College, Harbin, Heilongjiang, China

downwind drift decreased from 36% at 10 m to 3.7% at 50 m to 0.2% at 200 m across four trials at a wind speed of 0.4–0.9 km h⁻¹.³ Approximately one-third of the off-target loss occurred in the first 10–20 m downwind, with aerial drift deposits decreasing rapidly thereafter.

Glyphosate application frequency has increased concomitantly with the widespread adoption of GR crops. For example, the number of glyphosate applications has increased from 1 in 1997 (the year before GR corn was commercialized) to 1.3 in 2005 for corn; from 1 in 1995 (the year before GR soybean was commercialized) to 1.7 in 2006 for soybean; and from 1 in 1996 (the year before GR cotton was commercialized) to 2.2 in 2005 for cotton.⁴ GR crops are frequently planted adjacent to non-GR crops. Corn is usually planted in March–April, and soybean and cotton are planted in April–May in Mississippi. Thus, corn has greater potential for exposure to glyphosate drift from preplant applications than soybean and cotton. Glyphosate drift onto non-target crops from ground or aerial applications is common in the Mississippi Delta region. In 2008, 56 cases of herbicide drift onto non-target crops were reported in Mississippi, an increase of 21 cases from 2007. Rice, *Oryza sativa* L. (38%), wheat, *Triticum aestivum* L. (18%), soybean (9%), cotton (5%) and other Mississippi crops (30%) were impacted by herbicide drift in 2008. Thirty-three of the 56 Mississippi drift complaints in 2008 were due to glyphosate ground and aerial applications (Campbell J, Mississippi Department of Agriculture and Commerce, private communication, 2009). Simulated glyphosate drift injury has been reported in corn,^{5–7} soybean,^{8,9} rice^{7,10} and peanut, *Arachis hypogaea* L.¹¹ Although drift rates are typically sublethal, the injury can be severe in sensitive crops, depending on growth stage, and could reduce yields.^{5–7,12}

Numerous studies have examined the effect of droplet size, drift distance and spray deposition from pesticide applications,^{1–3,13} but few studies have determined the biological response of sensitive plant species to glyphosate drift. Information is lacking on how sensitive species will respond biologically to aerial glyphosate drift. This study examines the biological responses to aerial glyphosate drift in non-GR corn. Specific objectives of this research were to determine the effect of aerial glyphosate drift from a single swath of 18.3 m at a rate of 866 g ha⁻¹ on injury, chlorophyll, shikimate, plant height and shoot dry weight in non-GR corn.

2 MATERIALS AND METHODS

2.1 Experimental site and aerial application

An aerial application study was conducted in 2009 at the US Department of Agriculture–Agricultural Research Service, Crop Production Systems Research Farm, Stoneville, MS (33° 26' N, 90° 55' W) to determine injury and biological responses to glyphosate drift in non-GR corn. The field was tilled, and raised seedbeds spaced 102 cm apart were prepared prior to planting. The experimental area was treated with glufosinate at 0.45 kg Al ha⁻¹ plus pendimethalin at 1.12 kg Al ha⁻¹ prior to planting to kill existing vegetation and to provide residual weed control. Non-GR corn hybrid 'Pioneer 31P41' at 75 000 seeds ha⁻¹ was planted on 23 July 2009. Corn was planted in eight rows spaced 102 cm apart and 80 m long with four replications.

Aerial application of glyphosate was made over corn on 12 August 2009. At application, the corn was at the four-leaf stage. Glyphosate spray solution was prepared using glyphosate-potassium salt 540 g AE L⁻¹ (Roundup WeatherMax®; Monsanto

Co., St Louis, MO) at a rate of 866 g AE ha⁻¹ and 2.6 g rubidium chloride (RbCl) tracer in the tank mixture to allow proportional estimates of glyphosate concentrations downwind. Determination of glyphosate by liquid chromatography–mass spectrometry (LC-MS) is tedious and expensive, and therefore stable tracers have been sought to provide data for relative concentration where direct analysis of glyphosate is not feasible. RbCl is typically used as a non-invasive biomarker and has shown good stability as a tracer in drift and deposition studies. It is non-competitive with other potential chemical reactions and has provided repeatable results using atomic absorption spectrometry.¹³ Duffus¹⁴ classified Rb as an s-block alkali metal ion that naturally forms a weak complex and demonstrates insignificant oxidation–reduction reactions. Other elements in this category include Na, K and Ca. Chelates such as those in the p-block group that tend to bind strongly with sulfur¹⁴ can cause environmental pollution, but, to the present authors' knowledge, RbCl in trace amounts has not been documented to be environmentally deleterious.

An Air Tractor 402B spray airplane equipped with 54 CP-09 spray nozzles (CP Products, Tempe, AZ) set at a 5° downward deflection angle was used for application. The aircraft was calibrated to deliver 46.8 L ha⁻¹ at a release height of 3.7 m with an operating speed of 225 km h⁻¹ over an 18.3 m wide swath. One spray run in the west to east direction in the center of the field perpendicular to crop rows was flown over a marked swath line (Fig. 1). On-site weather conditions were recorded during the 4 s flight. The average wind speed was 11.2 km h⁻¹ from the northeast direction at an average of 64° from true north. Average air temperature was 28.5 °C and relative humidity was 72%, as determined during the spray run using a tripod-mounted Kestrel 4500 weather tracker (Nielsen-Kellerman, Boothwyn, PA).

The spray downwind drift lines were established along the crop rows and were perpendicular to the spray swath (Fig. 1). Sample locations were marked at 3, 6, 11, 15.8, 25.6 and 35.4 m, as measured from the downwind edge of the 18.3 m wide swath. Although this was a near-field drift study, sample locations were set to approximate a logarithmic spacing as specified by the Spray Drift Task Force.¹ One sampler was also located in the center of the spray swath (0 m) to represent highest exposure to glyphosate, and one upwind sampler was placed at 35.4 m north as measured from the upwind edge of the 18.3 m wide swath to serve as a control (corn not exposed to glyphosate) for comparison of biological responses to drift. These locations were established in all four replications. The biological data were collected from all eight rows in a 0.5 m wide band centered over the sampling locations.

The drift sampling stations were marked in one line within the second replication, parallel to corn (north to south) at 0–45 m distance from the flight line in both the downwind and upwind directions. Additional downwind drift sampling stations were set up 45° southwest and 45° southeast to the north–south sampling line. Mylar sampling sheets (12.7 cm × 13.0 cm) were placed on stands at corn canopy level at each station. Field personnel collecting Mylar sampling sheets wore latex gloves (changed after every run) and used toothpicks to slide Mylar from the stands into leak-proof-zipper bags. This was done to ensure no human contamination of sampling sheets. In the laboratory, each sampling sheet was rinsed with 1% HNO₃. Samples of RbCl were then analyzed using a Perkin Elmer AAnalyst 600 atomic absorption spectrometer (Perkin Elmer, Waltham, MA), which has a practical quantitation limit (PQL) of 0.7 ppb. A Mylar recovery test had been performed by applying ten drops of a 500 µL

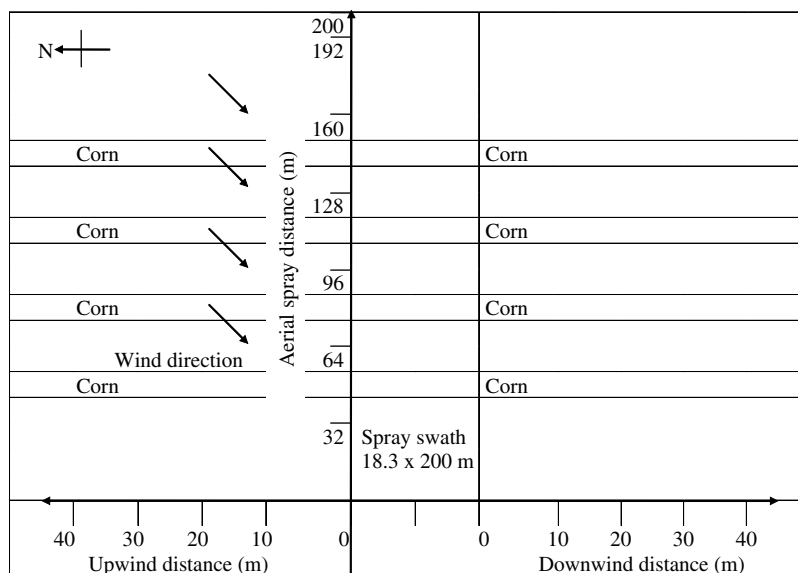


Figure 1. Schematic layout of the field for the glyphosate aerial drift study, Stoneville, MS.

spiked solution of RbCl to ten Mylar sheets, replicated twice. The solution was allowed to evaporate, and the sheets were then rinsed with 1% HNO₃ before analysis on the atomic absorption spectrometer. The mean concentration of RbCl recovered was 26.37 µg L⁻¹ as compared with 28.68 µg L⁻¹ spiked solution; an estimated recovery of 92%.

2.2 Corn injury and plant height

Corn injury was visually estimated at each sampling location, based on chlorosis, necrosis, stunted growth and plant death on a scale of 0 (no corn injury) to 100% (corn death). Corn injury was estimated on a weekly basis up to 3 weeks after application (WAA). Corn height was recorded on five randomly selected plants at 2 and 3 WAA. Plant height data were expressed as percentage reduction compared with the no glyphosate exposure control (upwind 35.4 m sampling location).

2.3 Chlorophyll and shoot dry weight determination

The youngest fully expanded leaf from three plants was sampled randomly at each location for chlorophyll determination. Chlorophyll was extracted with 10 mL dimethyl sulfoxide and quantified spectrophotometrically as described previously.¹⁵ Ten corn plants were excised at the soil surface and oven dried (60 °C, 72 h), and dry weights were recorded. Chlorophyll and shoot dry weight were determined at 1, 2 and 3 WAA, and data were expressed as percentage reduction compared with the no glyphosate control (upwind 35.4 m sampling location).

2.4 Shikimate determination

One disc (6 mm diameter) per leaf was sampled using a standard paper hole-punch adjacent to the midrib from the youngest fully expanded leaves from plants that were collected as described in Section 2.3 for shoot dry weight determination. Shikimate was determined spectrophotometrically following the protocols described previously, with modifications.^{16,17} Leaf discs were placed in screw-top 7 mL plastic vials and stored in a freezer until analyzed (about 4–6 weeks). A quantity of 1 mL of 0.25 M HCl was added to each vial and incubated at room temperature

for 90 min. A 25 µL aliquot of solution in duplicate was placed in a 96-well flat-bottom microtitre plate containing 100 µL of 0.25% periodic acid/0.25% meta-periodate solution and incubated for 60 min at room temperature. A 100 µL aliquot of 0.6 M sodium hydroxide/0.22 M sodium sulfite solution was added to each well, and absorbance was determined at 380 nm using a microplate reader (Synergy HT; BIO-TEK Instruments, Inc., Winooski, VT). Shikimate was quantified using a standard curve generated from known concentrations of shikimate, and data were expressed as percentage increase over the no glyphosate control (upwind 35.4 m sampling location).

2.5 Statistical analysis

Sampling location was considered as an independent variable, while biological responses were considered as dependent variables. Data were fitted to a sigmoidal logistic model to relate percentage injury, chlorophyll, shikimate, plant height and shoot dry weight reduction (y) to downwind drift distance (x):

$$y = \frac{a}{1 + \exp^{-(x-x_0)/b}} \tag{1}$$

where a is the difference of the upper and lower response limits (asymptotes), x₀ is the downwind drift distance that results in a 50% reduction in y (LD₅₀) and b is the slope of the curve around x₀. The regression parameters for equation (1) were computed using SigmaPlot® v.10.0 (Systat Software Inc., San Jose, CA). Regression parameters for drift distance–response curves were fitted to equation (1) and are presented in Table 1 (see Section 3.4).

The percentage of applied glyphosate deposited at each downwind sampling station was estimated in the southern direction by numerically integrating the amounts of RbCl tracer detected on Mylar sheets between sampling stations. This assumes that the relative percentage of RbCl tracer in the spray mix would be equivalent to the proportion of applied glyphosate in the same mixture. The integration was accomplished in a two-step process. First, amounts in the swath beginning at the demarcation (first station showing damage, station 3) were numerically

Table 1. Glyphosate LD₅₀ distance and estimated regression parameters from response curves fitted to equation (1)

Parameter	Time after aerial application (weeks)	Minimum (%)	Maximum (%)	Hillslope (% m ⁻¹)	LD ₅₀ (m)	R ²
Corn injury	1	8.84 (11.011) ^a	96.68 (2.867)	3.37 (0.925)	17.95 (2.006)	0.99
	2	-10.07 (16.221)	100.20 (2.099)	3.02 (0.593)	22.52 (2.769)	0.99
	3	-7.96 (4.489)	99.55 (0.493)	8.31 (1.442)	26.09 (0.345)	0.99
Chlorophyll	1	-8.59 (11.242)	98.98 (5.946)	2.84 (0.960)	12.22 (1.460)	0.99
	2	-4.68 (4.806)	101.31 (2.730)	3.83 (0.692)	13.43 (0.615)	0.99
	3	-17.43 (26.737)	98.55 (4.947)	3.72 (1.476)	21.57 (3.834)	0.98
Shoot dry weight	1	-3.20 (4.050)	67.14 (1.076)	3.13 (0.400)	17.29 (0.929)	0.99
	2	-28.73 (14.843)	81.94 (1.277)	2.75 (0.380)	25.11 (2.831)	0.99
	3	8.66 (2.016)	85.43 (0.836)	7.03 (0.670)	19.42 (0.459)	0.99
Plant height	1	DNC ^b	DNC	DNC	DNC	DNC
	2	-18.22 (30.014)	102.37 (3.953)	2.22 (0.705)	20.43 (5.383)	0.99
	3	-3.41 (10.818)	99.50 (1.690)	3.84 (0.633)	23.20 (1.725)	0.99
Shikimate	1	-44.00 (61.764)	342.50 (23.291)	2.30 (0.874)	13.11 (2.46)	0.98
	2	ND ^c	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND

^a Values in parentheses denote the standard error of the fitted value.

^b DNC, no data were collected at that sampling time.

^c ND, insufficient data due to plant mortality at aerial drift distances ranging from 0 to 20 m.

integrated between in-swath sampling station distances. Then, RbCl concentrations downwind from the edge of the defined spray swath were fitted to equation (2) using CurveExpert 1.36 (Daniel Hyams, Starkville, MS). Amounts at each downwind station determined from the model fit were also numerically integrated between sampling stations. Integrated amounts in-swath and downwind were then added together. The proportion of sprayed quantity as a function of distance could then be determined by dividing the amounts at each distance (sampling station) by the total amount over all stations:

$$y = \frac{1}{a \times x + b} \quad (2)$$

where y is the RbCl concentration, x is the downwind drift sampling distance, a is the slope of the curve and b is the intercept. Regression parameters for equation (2) were: $a = 0.020730049$; $b = 0.070218617$; $R^2 = 0.99$.

3 RESULTS AND DISCUSSION

3.1 Corn injury

Corn was unaffected in the upwind direction from the edge of the spray swath, but injury was observed downwind of the spray swath. Injury symptoms included chlorosis of the youngest leaves, necrosis, stunted growth and plant death. Corn was killed under the spray swath within a week (Fig. 2). At 1 WAA, corn was killed at 3 m from the edge of the spray swath, and corn injury decreased gradually with increasing distance to 18% at 35.4 m downwind of the spray swath. A similar trend was observed at 2 WAA, except that corn was killed up to 6 m. At 3 WAA, corn injury was exacerbated between 11 and 25.6 m, and corn recovered from injury at 35.4 m.

3.2 Chlorophyll

Chlorophyll reduction of 100% indicates that corn was dead, for example, at 3 m 1 WAA (Fig. 3). At 1 WAA, chlorophyll reduction decreased from 78% at 6 m to 22% at 15.8 m, with chlorophyll

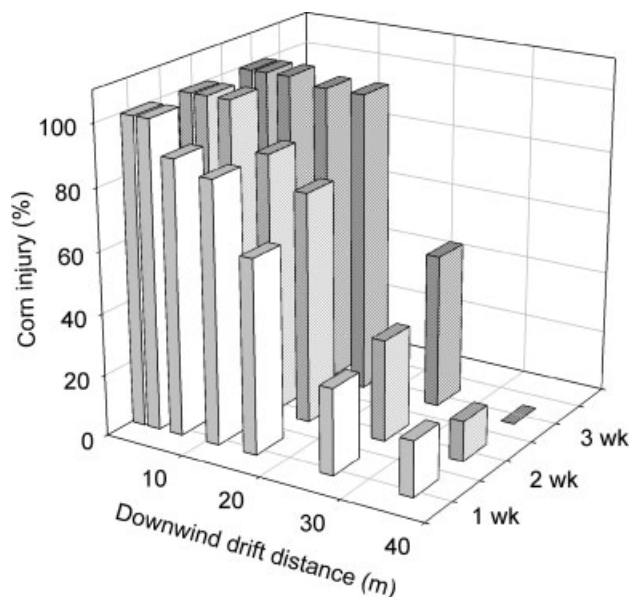


Figure 2. Visual estimates of corn injury at 1 and 3 weeks after glyphosate aerial application at Stoneville, MS, 2009. Observed mean values are plotted. Standard error bars have been excluded for increased clarity and trend quality.

content unaffected beyond 25.6 m. A similar trend was observed at 2 and 3 WAA. Overall, based on chlorophyll content, corn was unaffected from glyphosate drift beyond 25.6 m. Glyphosate is known to cause chlorophyll reduction in other plant species.^{18,19}

3.3 Shikimate

Shikimate was present in corn both upwind and downwind of the spray swath, with shikimate levels ranging from 15.3 ng mL⁻¹ at 35.4 m upwind to 68.7 ng mL⁻¹ at 0 m (the edge of the spray swath) at 1 WAA. Shikimate levels are elevated in plant species exposed to glyphosate.²⁰⁻²² Glyphosate inhibits the

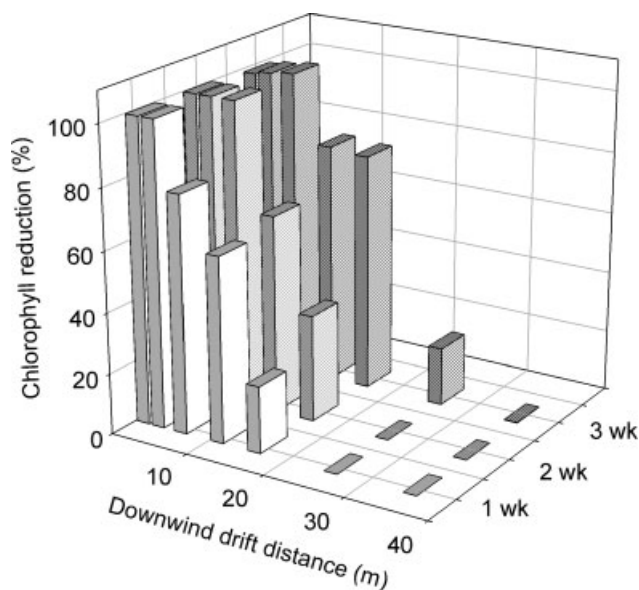


Figure 3. Chlorophyll reduction in corn at 1, 2 and 3 weeks after glyphosate aerial application at Stoneville, MS, 2009. Data are expressed as the percentage reduction compared with the no glyphosate exposure control. Observed mean values are plotted. Standard error bars have been excluded for increased clarity and trend quality.

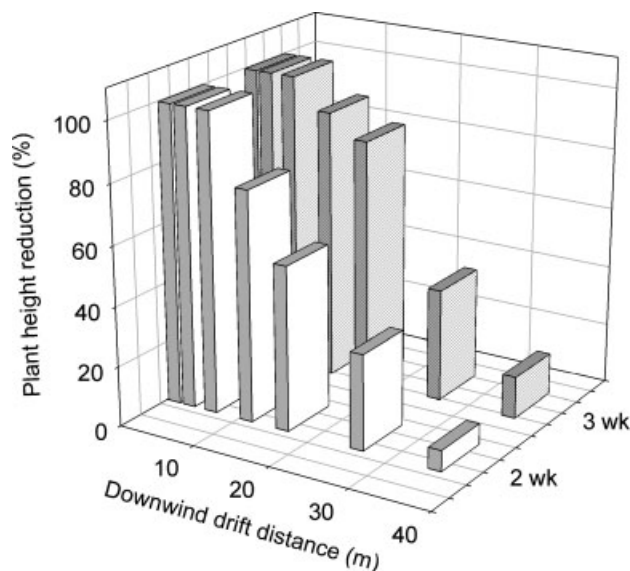


Figure 5. Corn plant height reduction due to drift at 2 and 3 weeks after glyphosate aerial application at Stoneville, MS, 2009. Data are expressed as the percentage reduction compared with the no glyphosate exposure control. Observed mean values are plotted. Standard error bars have been excluded for increased clarity and trend quality.

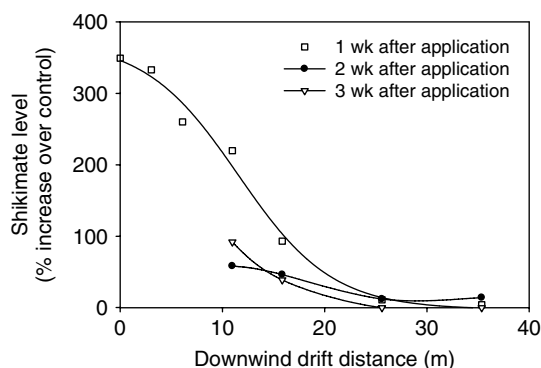


Figure 4. Elevated shikimate levels in corn owing to drift at 1, 2 and 3 weeks after glyphosate aerial application at Stoneville, MS, 2009. Because of corn death, no data were collected at locations proximal to the spray swath at 2 and 3 weeks after application. Data are expressed as the percentage increase over the no glyphosate exposure control. Observed mean values are plotted. One week after application data were fitted to equation (1). Standard error bars have been excluded for increased clarity and trend quality.

enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway.²³ By blocking EPSPS, glyphosate causes many-fold increases in shikimate levels in glyphosate-treated plants.²⁴ At 1 WAA, the shikimate level in corn was 349% higher at 0 m compared with the shikimate level in corn at 35.4 m upwind (Fig. 4). Shikimate decreased gradually from 333% at 3 m to 93% at 15.8 m, with levels unaffected beyond 25.6 m. At 2 and 3 WAA, shikimate was not measured at sample locations ≤ 6 m, as corn was dead and shikimate levels at sampling locations ≥ 15.8 m were lower than at 1 WAA. Overall, shikimate levels in corn rose in a greater proportion at 1 WAA than at 2 and 3 WAA. It is not uncommon for shikimate levels to rise immediately following glyphosate treatment and then to decrease to levels similar to those in non-treated plants over time.^{9,25,26}

3.4 Plant height and shoot dry weight

Corn exhibited stunted growth due to glyphosate drift (Fig. 5). Corn plant height was recorded only at 2 and 3 WAA, and a height reduction of 100% indicated that corn was dead. Plant height reduction decreased gradually with increasing downwind distance. At a distance of 35.4 m, corn height was reduced by 7% 2 WAA and by 14% 3 WAA. Corn shoot dry weight reduction was highest at a downwind distance of ≤ 6 m, regardless of the sampling date, and it should be noted that these weights represent dead plants (Fig. 6). Shoot dry weight reduction decreased gradually with increasing downwind distance. At 35.4 m, corn shoot dry weight was reduced by 4% 1 WAA, by 2% at 2 WAA and by 10% 3 WAA.

These data indicate that plant injury, chlorophyll, shikimate, plant height and plant dry weight due to glyphosate drift decreased with increasing downwind distance from the edge of the spray swath. Not all plant species are equally sensitive to glyphosate. The GR₅₀ rate, the rate required to cause a 50% reduction in plant growth, is commonly used to compare the differences in glyphosate sensitivity among plant species. The glyphosate GR₅₀ rate for non-GR corn is 93 g ha⁻¹.²¹ Similarly, different biological responses to glyphosate drift can be compared using a lethal distance (LD) value. The LD₅₀ is the distance that drift must travel to cause a 50% reduction in biological response. In the present study, the observed data were fitted to equation (1) to determine the LD₅₀ (Table 1). The LD₅₀ ranged from 12.22 to 17.95 m for corn injury, chlorophyll, shoot dry weight and shikimate at 1 WAA. At 3 WAA, the LD₅₀ ranged from 13.11 to 26.09 m for corn injury, chlorophyll, shoot dry weight and shikimate. It is sufficient to say that, overall, LD₅₀ ranged from 12.22 to 26.09 m for these parameters. Taking the GR₅₀ rate and the LD₅₀ distance together suggests that at least 10.7% (= 93 g ha⁻¹) of applied glyphosate was deposited between distances of 12 and 26 m.

The percentage deposited glyphosate as inferred by integrated amounts of RbCl between 12 and 26 m distances in the southern direction was 5.6%. Mylar samplers at each sampling station

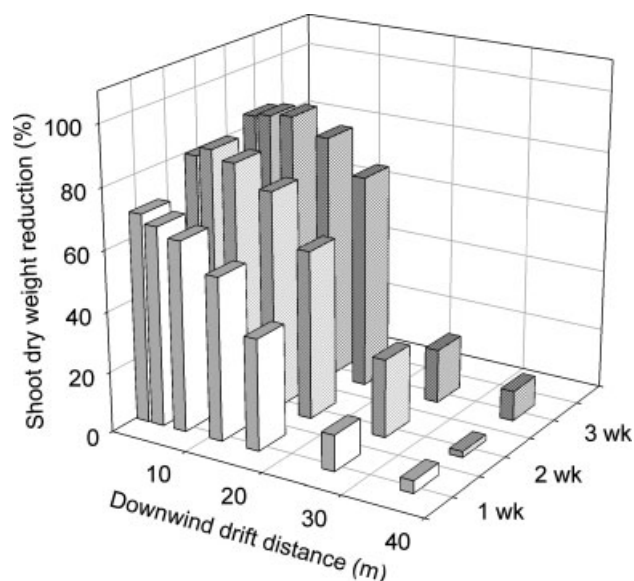


Figure 6. Corn shoot dry weight reduction due to drift at 1, 2 and 3 weeks after glyphosate aerial application at Stoneville, MS, 2009. Data are expressed as the percentage reduction compared with the no glyphosate exposure control. Observed mean values are plotted. Standard error bars have been excluded for increased clarity and trend quality.

were processed in triplicate, and the mean concentration was $16.3 \mu\text{g L}^{-1}$. The average standard deviation of concentration in the swath was $0.19 \mu\text{g L}^{-1}$, and in the downwind direction it was $0.036 \mu\text{g L}^{-1}$. The distance range to give 10.7% of applied glyphosate using integrated deposition from Mylar samplers was between 6 and 12 m downwind. It should be noted that proportions derived using spray samplers are subject to wind effects, turbulence and corresponding irregularities in the amount of material on samplers in the spray swath. For purposes of comparison with integrated deposition in the southern direction, the percentage applied between the 12 and 26 m distances in the southwest direction was calculated as 8.9%. This higher amount would be expected, as the samplers in this direction were directly in line with the prevailing wind. This compares well with the GR₅₀ and LD₅₀ derived value of 10.7%.

In summary, corn injury and other biological responses point to the same conclusion, that is, injury from glyphosate aerial drift is highest at the edge of the spray swath and decreases gradually with increasing distance. The glyphosate drift effect was lowest (0–18% corn injury, 2–10% dry weight reduction) at 35.4 m. The LD₅₀ distance, a measure of sensitivity to glyphosate, ranged from 12 to 26 m among the biological parameters. The visual injury estimation was rapid and non-destructive and as reliable as the chlorophyll, shikimate, plant height and plant dry weight determination method. The elevated shikimate level is unique to glyphosate exposure and is used as an early and highly sensitive indicator of the glyphosate effect on plants. However, with the widespread adoption of GR crops, it will be challenging to find a site free from glyphosate drift from neighboring fields. The LD₅₀ is a valuable indicator of potential corn yield loss. Corn injury of 45–55% has reduced the corn yield by as much as 49–56% in other studies.^{6,12} As corn injury from glyphosate aerial drift is evident, many strategies could be used to mitigate off-target drift. Aerial drift reduction strategies such as the use of low-drift flat-fan nozzles or rotary atomizers, the addition of adjuvants that increase droplet size, spray release at proper height, maintaining the maximum

distance possible from the sensitive crop and application during low wind speed and wind direction away from the sensitive crop could potentially minimize levels of near-field drift.

4 DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

ACKNOWLEDGEMENT

The authors thank Efren Ford, Earl Gordon, Terry Newton, Roger Bright, Earl Franklin, David Thornton, Linwood Roberts, Phelesia Foster, Cuwanda Flowers and Shawana Cox for field and laboratory technical assistance. Thanks also to the pilot, David Poythress, for his help in the field and flying the airplane.

REFERENCES

- Bird SL, Esterly DM and Perry SG, Off-target deposition of pesticides from agricultural aerial spray application. *J Environ Qual* **25**:1095–1104 (1996).
- Kirk IW, Aerial spray drift from different formulations of glyphosate. *Trans ASAE* **43**:555–559 (2000).
- Payne NJ, Spray dispersal from aerial silvicultural glyphosate applications. *Crop Prot* **12**:463–469 (1993).
- Agricultural Chemical Database*. [Online]. National Agricultural Statistics Service, United States Department of Agriculture. Available: <http://www.pestmanagement.info/nass/> [9 March 2010].
- Buehring NW, Massey JH and Reynolds DB, Shikimic acid accumulation in field-grown corn (*Zea mays*) following simulated glyphosate drift. *J Ag Food Chem* **55**:819–824 (2007).
- Brown LR, Robinson DE, Young BG, Loux MM, Johnson WG, Nurse RE, *et al*, Response of corn to simulated glyphosate drift followed by in-crop herbicides. *Weed Technol* **23**:11–16 (2009).
- Ellis JM, Griffin JL, Linscombe SD and Webster EP, Rice (*Oryza sativa*) and corn (*Zea mays*) response to simulated drift of glyphosate and glufosinate. *Weed Technol* **17**:452–460 (2003).
- Bellaloui N, Reddy KN, Zablotowicz RM and Mengistu A, Simulated glyphosate drift influences nitrate assimilation and nitrogen fixation in non glyphosate-resistant soybean. *J Ag Food Chem* **54**:3357–3364 (2006).
- Ellis JM and Griffin JL, Soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) response to simulated drift of glyphosate and glufosinate. *Weed Technol* **16**:580–586 (2002).
- Koger CH, Shaner DL, Krutz LJ, Walker TW, Buehring N, Henry WB, *et al*, Rice (*Oryza sativa*) response to drift rates of glyphosate. *Pest Manag Sci* **61**:1161–1167 (2005).
- Lassiter BR, Burke IC, Thomas WE, Pline-Srnić WA, Jordan DL, Wilcut JW, *et al*, Yield and physiological response of peanut to glyphosate drift. *Weed Technol* **21**:954–960 (2007).
- Reddy KN, Bellaloui N and Zablotowicz RM, Glyphosate effect on shikimate, nitrate reductase activity, yield, and seed composition in corn. *J Ag Food Chem* **58**:3646–3650 (2010).
- Smith LA, Mulrooney JE and Elmore CD, Experimental design and sampling techniques for effective evaluation of spray drift. ASABE paper no. 001029, American Society of Agricultural and Biological Engineers, St Joseph, MI, 18 pp. (2000).
- Duffus JH, 'Heavy metals' – a meaningless term? *Pure Appl Chem* **74**:793–807 (2002).
- Hiscox JD and Israelstam GF, A method for the extraction of chlorophyll from leaf tissues without maceration. *Can J Bot* **57**:1332–1334 (1979).
- Burke IC, Reddy KN and Bryson CT, Pitted and hybrid morningglory accessions have variable tolerance to glyphosate. *Weed Technol* **23**:592–598 (2009).
- Koger CH, Shaner DL, Henry WB, Nadler-Hassar T, Thomas WE and Wilcut JW, Assessment of two nondestructive assays for detecting

- glyphosate resistance in horseweed (*Conyza canadensis*). *Weed Sci* **53**:438–445 (2005).
- 18 Reddy KN, Hoagland RE and Zablotowicz RM, Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosate-resistant and susceptible soybean (*Glycine max*) varieties. *J New Seeds* **2**:37–52 (2000).
 - 19 Nandula VK, Reddy KN, Rimando AM, Duke SO and Poston DH, Glyphosate-resistant and -susceptible soybean (*Glycine max*) and canola (*Brassica napus*) dose response and metabolism relationships with glyphosate. *J Ag Food Chem* **55**:3540–3545 (2007).
 - 20 Reddy KN, Rimando AM and Duke SO, Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *J Ag Food Chem* **52**:2125–2130 (2004).
 - 21 Reddy KN, Rimando AM, Duke SO and Nandula VK, Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. *J Ag Food Chem* **56**:2125–2130 (2008).
 - 22 Pline WA, Wilcut JW, Duke SO, Edmisten KL and Wells R, Accumulation of shikimic acid in response to glyphosate applications in glyphosate-resistant and conventional cotton (*Gossypium hirsutum* L.). *J Ag Food Chem* **50**:506–512 (2002).
 - 23 Duke SO, Glyphosate, in *Herbicides: Chemistry, Degradation, and Mode of Action*, ed. by Kearney PC and Kaufman DD. Marcel Dekker, New York, NY, pp. 1–70 (1988).
 - 24 Lydon J and Duke SO, Glyphosate induction of elevated levels of hydroxybenzoic acids in higher plants. *J Ag Food Chem* **36**:813–818 (1988).
 - 25 Henry WB, Koger CH and Shaner DL, Accumulation of shikimate in corn and soybean exposed to various rates of glyphosate. Online. *Crop Manag* DOI: 10.1094/CM-2005-1123-01-R5 (2005.). Available: <http://plantmanagementnetwork.org/pub/cm/research/2005/shikimate/> [15 July 2005].
 - 26 Henry WB, Shaner DL and West MS, Shikimate accumulation in sunflower, wheat, and proso millet after glyphosate application. *Weed Sci* **55**:1–5 (2007).