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Biological Response of Soybean and Cotton to Aerial Glyphosate Drift

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*When glyphosate is applied to glyphosate-resistant (GR) crops, drift on to off-target sensitive crops may cause injury and mortality. An aerial application drift study was conducted in 2009 to determine biological effects of glyphosate on non-glyphosate-resistant (non-GR) cotton (*Gossypium hirsutum *L.) and non-GR soybean [*Glycine max *(L.) Merr.]. Glyphosate at 866 g ae/ha was applied using an Air Tractor 402B agricultural aircraft in an 18.3 m spray swath to crops at the two- to three-leaf stage. Visual plant injury, chlorophyll, shikimate, plant height, and shoot dry weight were determined at one, two, and three weeks after application (WAA) of glyphosate. Biological responses differed between crops as a function of downwind drift distance. For example, at 3 WAA soybean was dead 6 m downwind from the spray swath, whereas cotton sustained 85% visual injury. Plant injury was not observed*

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beyond 25.6 m downwind in soybean and 35.4 m downwind in cotton at 3 WAA. Chlorophyll reduction was higher (80%) in soybean compared with cotton (43%) at 0 m from the edge of the spray at 1 WAA. Shikimate levels 1 WAA decreased from 1518% at 0 m to 209% at 35.4 m downwind in soybean; at the same sampling time shikimate levels in cotton decreased from 464% at 0 m to 0% at 35.4 m. At 35.4 m downwind, shoot dry weight (5–13%) and plant height (6–8%) were reduced in both crops at 3 WAA. The biological response of soybean and cotton to glyphosate drift decreased with increased distance from the edge of spray swath. These biological data suggested that soybean was more susceptible to glyphosate drift than cotton and elevated shikimate level could be used as a sensitive indicator to confirm plant exposure to glyphosate drift.

KEYWORDS *Aerial application, drift injury, glyphosate, off-target, shikimate*

INTRODUCTION

Total glyphosate use and application frequency have proliferated within a year with the adoption of glyphosate-resistant (GR) cropping systems. In Mississippi, the number of glyphosate applications per year has increased from 1.2 in 1995 (year before GR soybean was commercialized) to 2.6 in 2006 for soybean, and from 1.1 in 1996 (year before GR cotton was commercialized) to 3.1 in 2005 for cotton (NASS 2010). This trend has created an environment conducive to drift problems, particularly under extended rainy periods when wet soil conditions mandate aerial over-ground application methods. When glyphosate is applied to GR crops, drift on to non-GR crops may cause injury and reduce yields. Glyphosate drift onto non-target crops from ground or aerial applications is common in the Mississippi Delta region. In 2009, 30 cases of herbicide drift onto non-target crops were reported in Mississippi, with 24 of these complaints attributed to glyphosate (J. Campbell, Mississippi Department of Agriculture and Commerce, pers. communication 2010). Rice, *Oryza sativa* L. (70%), wheat, *Triticum aestivum* L. (7%), soybean (3%), cotton (3%), and other Mississippi crops (17%) were impacted by herbicide drift in 2009.

Glyphosate drift is a concern because the compound is a non-selective, systemic herbicide very toxic to sensitive plant species. Data for simulated glyphosate drift effects on corn, *Zea mays* L. (Ellis et al. 2003; Buehring, Massey, & Reynolds 2007; Brown et al. 2009; Reddy, Bellaloui, & Zablotowicz 2010), soybean (Ellis & Griffin 2002; Bellaloui et al. 2006), rice (Ellis et al. 2003; Koger et al. 2005b), and peanut, *Arachis hypogaea* L. (Lassiter et al. 2007), exist. A few studies, however, have explored glyphosate downwind drift deposition from aerial applications (Yates, Akesson, & Bayer

1978; Payne, Feng, & Reynolds 1990; Payne & Thompson 1992; Payne 1993; Kirk 2000; Reddy et al. 2010), with a few studies measuring biological effects on sensitive crops (Yates, Akesson, and & Bayer 1978; Reddy et al. 2010). Previously, we reported on the biological effects of glyphosate drift arising from aerial application on non-GR corn (Reddy et al. 2010). Herein, we report on the biological response, i.e., plant injury, chlorophyll content, shikimate accumulation, plant height, and shoot dry weight, of non-GR cotton and non-GR soybean as affected by downwind drift from aerial application of glyphosate at a rate of 866 g ae/ha and wind speed of 11.2 km/h.

MATERIALS AND METHODS

Experimental Conditions

An aerial application study was conducted in 2009 at the U.S. Department of Agriculture, Agricultural Research Service, Crop Production Systems Research Farm, Stoneville, MS, as described by Reddy et al. (2010). Briefly, the field was tilled and raised seedbeds were prepared prior to planting. The experimental area was treated with glufosinate at 0.45 kg ai/ha plus pendimethalin at 1.12 kg ai/ha before planting to kill existing vegetation and to provide residual weed control. Non-GR cotton cultivar 'FM955LL' at 100,000 seed/ha and non-GR soybean cultivar 'SO80120LL' at 285,000 seed/ha were planted on July 23, 2009. Each crop was planted in eight rows spaced 102-cm apart and 80-m long with four replications.

Glyphosate was applied using spray aircraft over the crops on August 12, 2009. At application, cotton was at two- to three-leaf stage and soybean was at two- to three-trifoliolate leaf stage. Glyphosate spray solution was prepared using commercial formulation of potassium salt of glyphosate (Monsanto, St. Louis, MO) at a rate of 866 g ae/ha. The maximum label rate for a single in crop application is 1,260 g/ha in cotton and 1,733 g/ha in soybean (Monsanto 2010). However, the glyphosate label specifically limits aerial application in cotton to 866 g/ha, hence this rate was used in the study. An Air Tractor 402B spray airplane equipped with 54 CP-09 spray nozzles (CP Products, Tempe, AZ) set at a 5-degree downward deflection angle was used for application. The aircraft was calibrated to deliver 46.8 L/ha at a release height of 3.7 m with an operating speed of 225 km/h over an 18.3-m-wide swath. One spray run west to east direction in the center of the field perpendicular to crop rows was flown over a marked swath line (Figure 1). On-site weather conditions were recorded during the 4-second flight. The average wind speed was 11.2 km/h from the northeast direction at an average of 64° from the True North. Mean air temperature was 28.5°C, and relative humidity was 72% as acquired during the spray run using a tripod mounted Kestrel 4500 weather tracker (Nielsen-Kellerman, Boothwyn, PA).

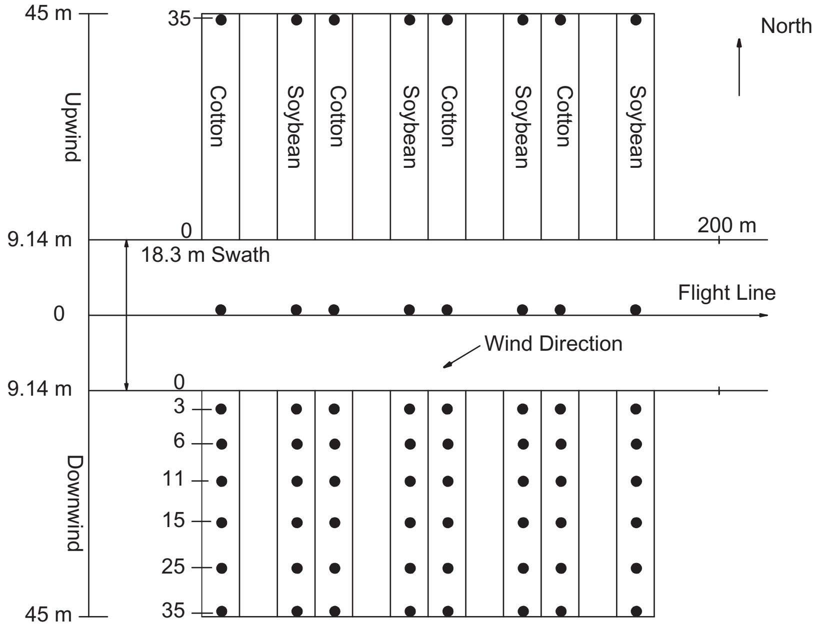

FIGURE 1 Schematic view of the experimental site and biological sampling locations for glyphosate aerial drift study in Stoneville, MS. Glyphosate was applied using an airplane flown over a marked swath of 18.3-m wide in the west to east direction in the center of the field perpendicular to crop rows. The dots indicate locations where biological samples were collected.

The spray downwind drift lines were established along the crop rows and were perpendicular to the spray swath (Figure 1). Sample locations were marked at 0, 3, 6, 11, 15.8, 25.6, and 35.4 m, as measured from the downwind edge of the 18.3 m wide swath. One upwind sample location at 35.4 m, as measured from the upwind edge of the 18.3 m wide swath, was included as a control (crops not exposed to glyphosate) for comparison of biological responses to drift. These locations were established in all four replications of each crop. The biological data were collected from all eight rows in a 0.5-m-wide band centered over the sampling location except at 0 m. For the 0 m sampling location, data were collected from the 18.3 m spray swath. The sampling location at 0 m represented the highest exposure to glyphosate, while the 35.4 m upwind sampling location represented no glyphosate exposure.

Plant Injury and Plant Height

Cotton and soybean injury was estimated visually at each sampling location based on chlorosis, necrosis, stunted growth, and plant death on a scale of 0 (no plant injury) to 100% (plant death). Plant injury was visually estimated at 1, 2, and 3 weeks after application (WAA). Cotton and soybean plant height was recorded on five randomly selected plants at 2 and 3 WAA. Plant height data were expressed as percent reduction as compared with no glyphosate-exposure control.

Chlorophyll

Chlorophyll content was determined using the youngest fully expanded leaf from three randomly sampled plants at each location at 1, 2, and 3 WAA. Chlorophyll was extracted with 10 mL dimethyl sulfoxide, and chlorophyll concentrations were quantified spectrophotometrically as described previously (Hiscox & Israelstam 1979).

Shikimate and Shoot Dry Weight

Ten plants were excised at the soil surface randomly from each sampling location, immediately transported to the laboratory, and assayed for shikimate content at 1, 2, and 3 WAA. One 6-mm-diameter disc per leaf was sampled adjacent to the midrib from the youngest fully expanded leaf from each of the 10 plants using a standard paper hole-punch. Shikimate levels were determined spectrophotometrically following the protocols described previously with modifications (Burke, Reddy, & Bryson 2009; Koger et al. 2005a). Briefly, leaf discs were placed in screw-top 7-mL plastic vials and stored in a freezer until analyzed (about 4 to 6 wk). One 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 read at 380 nm using a microplate reader (Biotek Synergy HT, Bio Tek Instruments Inc., Winooski, VT). Shikimate was quantified using a standard curve generated from known concentrations of shikimate, and the data expressed as percent increase over no glyphosate control.

After sampling leaf discs for shikimate determination, plants were oven-dried (60°C, 72 h), and dry weights were recorded. Shoot dry weight was determined at 1, 2, and 3 WAA, and data was expressed as percent reduction as compared with no glyphosate control (upwind 35.4 m sampling location).

Statistical Analysis

Sample locations at 0, 3, 6, 11, 15.8, 25.6, and 35.4 m downwind were regarded as treatments in each crop with four replications. The data from each crop were analyzed separately. The data were subjected to analysis of variance using PROC GLM (SAS software, release 8.2, Windows version 5.1.2600, SAS Institute, Cary, NC), and treatment means were separated at the 5% level of significance using Fisher's protected LSD test.

RESULTS AND DISCUSSION

Plant Injury

Glyphosate effects on soybean and cotton injury (chlorosis, necrosis, stunted growth, and plant death) were apparent within 1 WAA. Regardless of evaluation timing and crop, plant injury at 0 m was at least 75% but decreased to less than 23% at 35.4 m downwind (Figure 2). Generally, the plant-injury data indicated that soybean was more sensitive to glyphosate drift than cotton. For example, by 3 WAA, soybean was dead up to 6 m downwind from the edge of spray swath. Conversely, for that same time period and drift distance, cotton sustained 85% injury. Similar results were noted for glyphosate drift relative to corn injury (Reddy et al. 2010). In that study at 3 WAA, corn

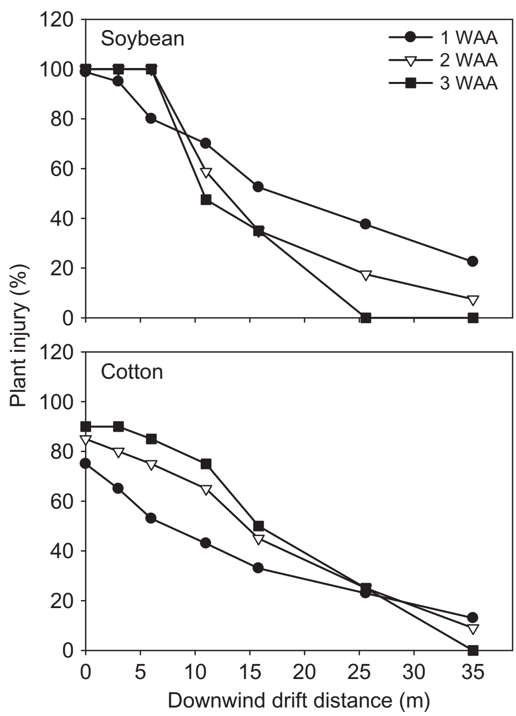

FIGURE 2 Visual estimates of soybean and cotton injury at 1, 2, 3 wk after glyphosate aerial application (WAA) at Stoneville, MS, 2009. LSD ($P = 0.05$) for comparing means within 1, 2, and 3 WAA were 18, 21, and 28, respectively in soybean, and 12, 13, and 23, respectively, in cotton.

was dead up to 15.8 m downwind, which likely indicated that corn was more sensitive to glyphosate drift than either soybean or cotton.

Shikimate

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase in the shikimate pathway (Duke 1988), which causes shikimate to accumulate in sensitive plant species (Lydon & Duke 1988). No other herbicide causes this effect, thus elevated shikimate levels in plants is diagnostic for glyphosate exposure (Buehring, Massey, & Reynolds 2007; Koger et al. 2005a; 2005b; Burke, Reddy, & Bryson 2009; Reddy et al. 2008). In our experiment, shikimate concentrations in soybean and cotton were highest within 1 WAA and declined for the next two sampling times (Figure 3), a trend consistent across various plant species when exposed to glyphosate (Koger et al. 2005b; Henry, Koger, & Shaner 2005; Henry, Shaner, & West 2007). Shikimate levels, regardless of crop or WAA, tended to decrease as downwind drift distance

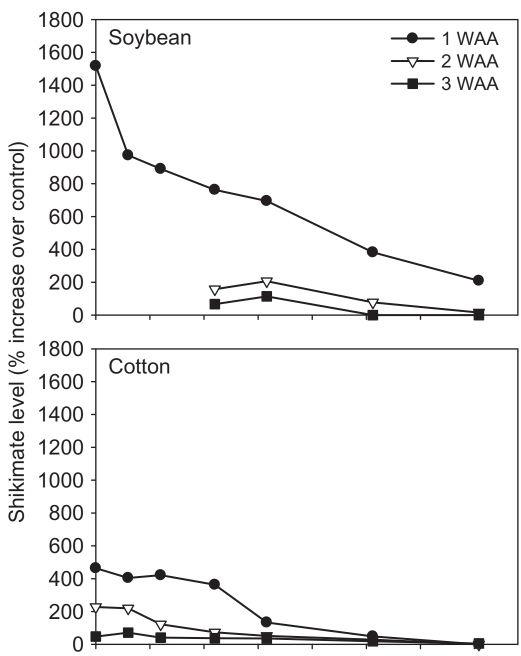

FIGURE 3 Elevated shikimate levels in soybean and cotton due to drift at 1, 2, 3 wk after glyphosate aerial application at Stoneville, MS, 2009. Due to soybean death, no data were collected at locations proximal to the spray swath at 2 and 3 wk after application. Data are expressed as percent increase over no glyphosate control. LSD ($P = 0.05$) for comparing means within 1, 2, and 3 WAA were 587, non-significant, and non-significant, respectively in soybean, and 276, 104, and 39, respectively, in cotton.

increased. This trend was more evident for the earlier sampling dates. For all distances less than 25.6 m, shikimate levels were generally higher in soybean than cotton.

Chlorophyll

Chlorophyll reductions were higher (80%) at 0 m from the edge of the spray swath and reduced to 2% at 25.6 m at 1 WAA (Figure 4). At 2 and 3 WAA, chlorophyll was not measured at downwind distance $\leq$ 6 m, as soybean plants were dead. Chlorophyll reductions at downwind distance $\geq$ 11 m in soybean were similar to chlorophyll levels at 1 WAA. In cotton, chlorophyll reduction was highest (43%) at 0 m and lowest (8%) at 25.6 m from the edge of the spray swath at 1 WAA (Figure 4). At 2 and 3 WAA, chlorophyll reductions were lower than at 1 WAA, with no significant differences among downwind sample locations within each sampling time. Overall, chlorophyll in cotton was less affected than soybean, which supported the visual

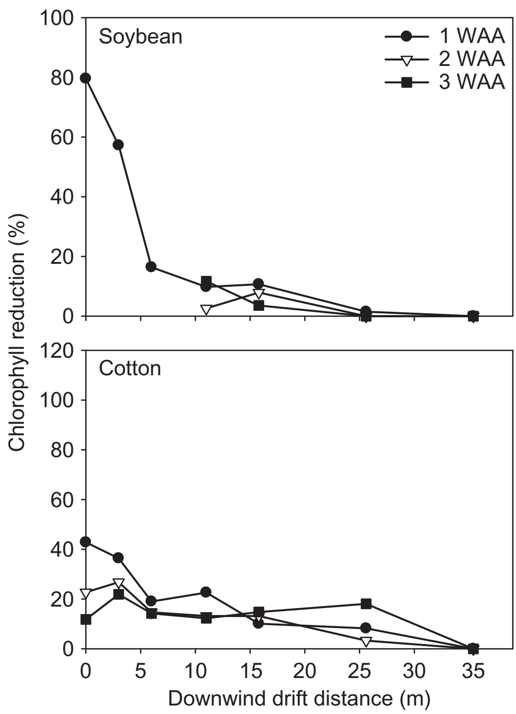

FIGURE 4 Chlorophyll reduction in soybean and cotton at 1, 2, 3 wk after glyphosate aerial application (WAA) at Stoneville, MS, 2009. Data are expressed as percent reduction as compared to no glyphosate control. LSD ($P = 0.05$) for comparing means within 1, 2, and 3 WAA were 39, non-significant, and non-significant, respectively in soybean, and 27, non-significant, and non-significant, respectively in cotton.

plant injury estimation that soybean was more sensitive to glyphosate than cotton.

Plant Height and Shoot Dry Weight

In soybean, plant height was not measured at sample locations ≤ 6 m because of mortality (Figure 5). At 2 WAA, soybean plant height was reduced from 39% at 11 m to 5% at 35.4 m distance. A similar trend was observed at 3 WAA. In cotton, glyphosate drift reduced plant height from 54% at 0 m to 16% at 35.4 m distance at 2 WAA. By 3 WAA, cotton plant height reduction increased near the spray swath compared with 2 WAA.

Soybean shoot dry weight reduction decreased from 48% at 0 m to 8% at 35.4 m distance at 1 WAA (Figure 6). At 2 and 3 WAA, soybean shoot dry weights at downwind distance of ≤ 6 m represented dead plants. At a distance of 35.4 m, soybean shoot dry weight was reduced by 5 to 8%, regardless of sampling time. In cotton, shoot dry weight reduction was ≤ 9% regardless of downwind distance at 1 WAA (Figure 6). Cotton shoot dry

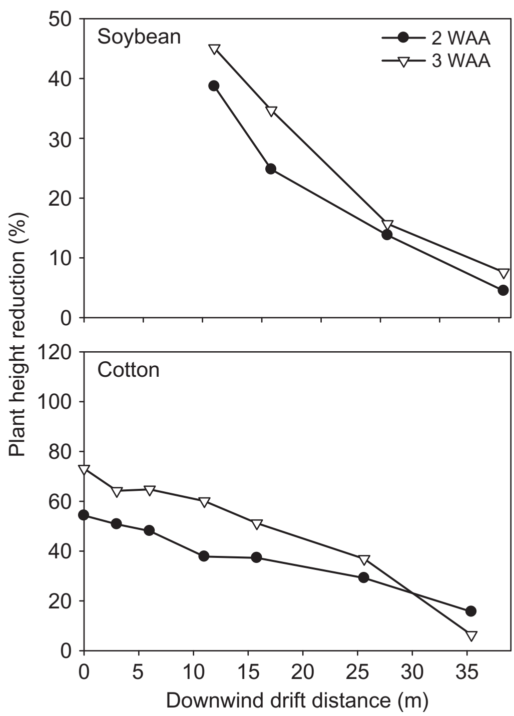

FIGURE 5 Soybean and cotton plant height reduction due to drift at 2 and 3 wk after glyphosate aerial application (WAA) at Stoneville, MS, 2009. Data are expressed as percent reduction as compared to no glyphosate control. LSD ($P = 0.05$) for comparing means within 2 and 3 WAA were 14 and 18, respectively in soybean, and 8 and 14, respectively in cotton.

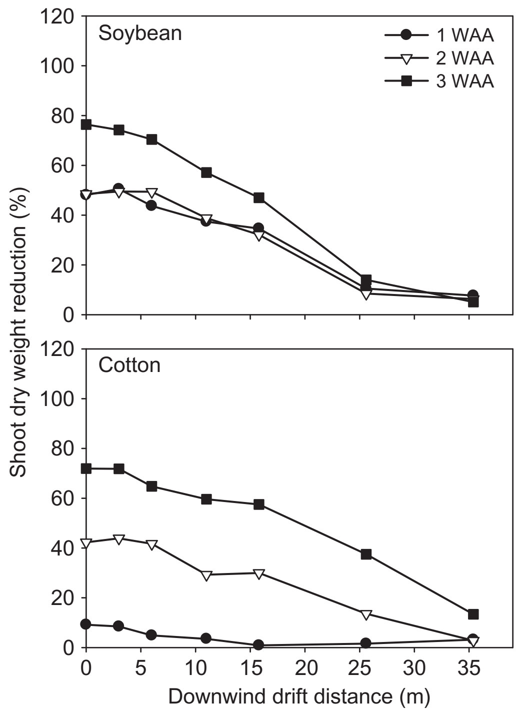

FIGURE 6 Soybean and cotton shoot dry weight reduction due to drift at 1, 2, 3 wk after glyphosate aerial application (WAA) at Stoneville, MS, 2009. Data are expressed as percent reduction as compared to no glyphosate control. LSD ($P = 0.05$) for comparing means within 1, 2, and 3 WAA were 17, 19, and 17, respectively in soybean, and non-significant, 17, and 23, respectively in cotton.

weight reduction levels at 2 WAA were higher than at 1 WAA, and levels at 3 WAA were higher than at 2 WAA on a relative basis. At a distance of 35.4 m, cotton shoot dry weight was reduced by 3% to 13%, regardless of sampling time.

These results indicate that biological responses to glyphosate drift in both soybean and cotton were severe at proximal to the spray swath but were minimal at 35.4 m downwind. At 6 m downwind from the spray swath, soybean plants were dead, whereas cotton sustained 85% injury at 3 WAA. At a downwind distance of 35.4 m, the glyphosate drift effect was lowest: 0% to 23% plant injury and 5% to 8% shoot dry weight reduction in soybean, and 0 to 13% plant injury and 3% to 13% shoot dry weight reduction in cotton, regardless of sampling time. The biological responses to glyphosate in soybean are similar to the responses reported previously in corn (Reddy et al. 2010). Overall, these biological responses to glyphosate drift suggested that soybean was more sensitive to glyphosate than cotton. Elevated shikimate level is unique to glyphosate and is a sensitive indicator of glyphosate effect on susceptible plants. Shikimate measurement coupled with plant injury, chlorophyll content, plant height, and plant dry weight data could be used as reliable indictors to confirm glyphosate exposure of susceptible plants.

Aerial application is the only convenient method of choice for farmers during extended rainy periods and wet soil conditions for timely glyphosate application. Numerous variables influence drift from aerial applications. Aerial applicators should carefully strive to select various aerial drift reduction strategies to minimize glyphosate drift onto off-target sensitive plant species.

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