Uptake, Translocation, and Dose Response of Postemergence Applied Diclosulam to Bristly Starbur (*Acanthospermum hispidum*)

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ABSTRACT

Laboratory and greenhouse studies were conducted to determine the absorption, translocation, dose response, efficacy, and effects on biomass of the herbicide diclosulam on bristly starbur (*Acanthospermum hispidum* DC.) In laboratory experiments, ^{14}C-diclosulam absorption and translocation was evaluated in bristly starbur. Greenhouse studies determined bristly starbur growth response to postemergence applied diclosulam at 0, 0.2, 0.4, 0.8, 1.6, 3.3, and 6.5 g ai/ha. Bristly starbur seed were collected from two Georgia locations, hand cleaned, and grown in the greenhouse. Bristly starbur plants were then foliar treated with diclosulam when plants were in the 4 to 6 leaf stage of growth. ^{14}C-diclosulam was applied to a single non-treated, most fully developed bristly starbur adaxial leaf, after the rest of the plant was foliar treated with 0.8 g ai/ha of diclosulam. At 24 and 48 hours after treatment, ^{14}C-diclosulam was translocated acropetally in bristly starbur to the plant apex, with little to no movement to the lower plant parts including lower leaves, stem and roots. For the dose response studies, bristly starbur growth reduction dry weight (GR_{50}) and efficacy (I_{50}) for diclosulam ranged from 0.95 to 0.92 g ai/ha, respectively. Based on these data, bristly starbur susceptibility to diclosulam was due to its translocation to apical growing points within 24 to 48 hours where acetoacetate synthase (ALS) inhibition occurred resulting in eventual plant death. While bristly starbur is controlled with diclosulam rates below the standard use rate of other weeds, the standard field rates in combination with other herbicide mechanisms of action will continue to be recommended to peanut growers to reduce the potential for bristly starbur ALS resistance selection.

Key Words: *Acanthospermum hispidum* DC, bristly starbur, diclosulam, dose response curves, ^{14}C-herbicide absorption and translocation.

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In the southeastern US production region of Alabama, Florida, and Georgia, weed control in peanut is accomplished with tactical measures of multiple herbicides with varying mechanism of action. This is accomplished via preemergence (PRE), at peanut emergence (AE), and postemergence (POST) herbicide applications. These timings are often dictated by the susceptibility of the weed species and crop safety.

Multiple applications of contact and residual herbicides are commonly used for peanut weed control as crop development and maturity require a long growing season (140-160 days). Diclosulam {*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]-triazolo[1,5-*c*]pyrimidine-2-sulfonamide} is an acetolactate synthase (ALS) inhibiting herbicide from the triazolopyrimidine sulfonanilide chemical family (Kleschick e*t al.*, 1992) registered since 2001 for peanut. Early research indicated that diclosulam provided effective weed control of dicot and nutsedge (*Cyperus* sp.) weed species in peanut when applied preplant incorporated (PPI), PRE, AE, or POST applications (Grey *et al.* 2001; Prostko *et al.*, 1999; Bailey *et al.*, 1998; Grichar *et al.*, 1998; Prostko *et al.*, 1998).

Historically, Benghal dayflower (*Commelina benhgalensis* L.) was not an issue in the southeastern US agronomic cropping systems. However, increased use of glyphosate in glyphosate-resistant crops from the mid-1990's caused a weed shift in these production systems such that Benghal dayflower was prevalent by the early 2000's (Webster *et al.* 2005; Webster and Nichols, 2012). Benghal dayflower is susceptible to several contact herbicides including paraquat, but this control is temporary in peanut due to lack of residual activity (Stephenson and Brecke, 2011). Benghal dayflower is also very fecund, producing seed from aerial and subterranean flowers that results in re-infestation, even after initial successful control in the same growing season (Sabila, *et al.* 2012; Webster *et al.* 2007).

University research noted that diclosulam, traditionally a PPI or PRE herbicide in peanut, could be used to control Benghal dayflower and other weeds when applied POST (Prostko *et al.,* 2004; Lancaster *et al.* 2007; Everman *et al.* 2006; Grichar 2007). With this knowledge and support from Dow AgroSciences, University of Georgia researchers investigated and obtained a section

24(c) registration for Georgia, and was subsequently granted for Florida and Mississippi for peanut (Anonymous 2013). Research on diclosulam in peanut indicated variable control of many weed species in North Carolina; diclosulam POST application timing and rates experiments indicated variable control of common ragweed (*Ambrosia artemisiifolia* L.) from 60 to 100%, morningglory (*Ipomoea* sp.) at 44 to 100%, and common lambsquarters (*Chenopodium album* L.) from 35 to 92% (Everman *et al.*, 2006; Lancaster *et al.*, 2007). Diclosulam applied in Georgia (Prostko *et al.*, 2004) and Texas (Grichar 2007) controlled Florida beggarweed [Desmodium tortuosum (Sw.) DC.] less than 77%, and it did not control sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby]. Palmer amaranth (*Amaranthus palmeri* S. Wats) control was variable (0 to 99%); this could be attributed to widespread ALS resistance in Georgia Palmer amaranth biotypes (Wise *et al.*, 2009). However, extension recommendations from Georgia, Florida, and Alabama indicate that bristly starbur is extremely sensitive (>95% control) to diclosulam POST applications (Prostko, *et al.* 2004).

In 2009, diclosulam was applied PPI, PRE, or POST to over 100,000 ha of peanut in the southeast US (NAWQA, 2012). While diclosulam POST resulted in variable weed control of some species, bristly starbur control was unique and consistently near 100% regardless of size. The physiological factors that impart the extreme sensitivity of bristly starbur to diclosulam are not known.

Bristly starbur is a C_3 plant native to tropical America, introduced to the US in the 1800's and is now distributed in over 40 countries. The Latin binomial, *Acanthospermum hispidum* DC, is descriptive of the plant; *Akanthos* is Greek for spine, thorn, or prickle, *sperma* means seed, and *hispidum* is Latin for bristly or rough (Holm *et al.*, 1997). The achene (also called a bur) is approximately 6 mm long, with stiff hooked spines, and two long spines at the apex (Figure 1). Seed are moved by clinging to equipment and animals, and both the vegetative parts of the plants and seed are poisonous to consume. In addition, the seed and plants are reported to contain allelochemicals (various phenolic acids) (Holm *et al.* 1997). Bristly starbur control is essential to prevent proliferation to other fields via contaminated harvesting equipment, peanut hay, and peanut seed given this species unique adapted ability to cling too many different surfaces. In 2009 and 2013 bristly starbur was the 6th and 10th most common and troublesome weed, respectively in Florida peanut and the 8th most common weed in Georgia peanut (Webster, 2009; 2013).

Figure 1. Bristly starbur (*Acanthospermum hispidum*) seed from Georgia (Photograph by Sidney Cromer, University of Georgia).

No reports of ALS-resistant bristly starbur have occurred. Target-site alteration as a mechanism of ALS-resistance in Palmer amaranth bitotypes from Georgia have been noted by substitutions at Trp574, Ser653, and Ala122 (Vencill *et al.*, 2013). Weeds develop resistance to ALS-herbicides due to their widespread usage in multiple crops, the affinity for strong selection pressure, extreme weed sensitivity, and low use rates. Mutations in ALS conferring herbicide resistance are at least partially dominant and because the gene is nuclear inherited; it can be transmitted by seed and pollen (Tranel and Wright, 2002). Given that ALS-herbicide resistance is very common in many different weed species, there is concern that using diclosulam at reduced rates POST could negatively select for potential ALS-resistant bristly starbur.

While growers have now used diclosulam for several years to control weeds PPI, PRE, and POST in peanut, there are several questions as to why this herbicide is so effective on bristly starbur and document this physiology. Therefore, the objectives of this study were to: 1) evaluate bristly starbur dose response to diclosulam rates to establish the GR_{50} and I_{50} in greenhouse experiments, and 2) compare the absorption and mobility of ^{14}C-diclosulam over time when applied POST to bristly starbur in laboratory experiments.

Materials and Methods

Multiple laboratory, greenhouse, and field experiments were conducted. Laboratory and greenhouse studies were conducted at the University of Georgia Weed Science Herbicide Research facilities

located on the College of Agriculture Experiment Station in Tifton, GA (31.48° N, -83.529° W).

Seed Collection. Seed were collected from two geographically separate populations in Georgia in Terrell County (31.69 N, -81.52 W) and in Tattnall County (32.02 N, -82.22 W). These represented populations from the piedmont and coastal area of the state, respectively. Bristly starbur plants were collected based on a visual assessment of plant maturity. After greater than 50% leaf drop, entire plants were cut at the soil surface, placed into nylon bags, and then dried in a forced air chamber with no supplemental heat. After drying, plant stems were stripped of the seed heads by hand, threshed over sieves, and the remaining chaff removed with forced air. Cleaned seed were then placed into glass vials, labeled with the appropriate GPS coordinates, placed in controlled environmental storage, where they remained until testing.

Greenhouse and Laboratory Experiments. Laboratory testing of bristly starbur seed germination indicated approximately 80% for the Terrell County population and 50% for Tattnall County. After germination testing, bristly starbur seed were sown into 10 by 10 cm pots filled with a topsoil mix, watered daily, and fertilized (Miracle-Gro, P.O. Box 606, Marysville, OH 43040) weekly. After two weeks, pots were thinned to one plant per pot, which constituted an experimental unit. Greenhouse temperatures were regulated with diurnal settings of 32/25 (± 5 C) and included supplemental light provided by metal halide growth lights for 16 h per day.

Diclosulam Dose Response. In greenhouse studies, bristly starbur in the 4th - to 6th-leaf growth stage was treated with a POST application of six rates of diclosulam (0.2, 0.4, 0.8, 1.6, 3.3, and at 6.5g ai/ha) on a logarithmic dose using a common multiplier of 2 (Seefeldt et al., 1995). A non-treated control was also included in the treatment structure. For each diclosulam rate, foliar treatments were applied using deionized water and a compressed air sprayer calibrated to deliver 140 L/ha at 165 kPa. Nonionic surfactant (NIS) (80-20 Chem Nut Inc., Leesburg, GA 31763) at 0.25% (v/v) was included with all POST treatments. After treatment, all plants were returned to the greenhouse and maintained as previously described. Experiments were conducted three times; and all studies consisted of 4 to 8 replications per treatment. Each experiment was arranged as a randomized complete block design. Visual estimates of bristly starbur control were recorded 14 and 21 days after treatment (DAT), using a scale of 0 (no control) to 100 % (plant death). Above ground biomass was then harvested

21 DAT, dried in paper coin envelopes at 50 C for 72 hours and biomass measured.

Data were subjected to ANOVA and evaluated for experimental trial by population origin interactions. All data for dose response experiments were then subjected to ANOVA. Bristly starbur visual estimates of control and dry weight data were regressed against diclosulam rate (SAS 1999). The log-logistic regression equation was used to describe the dose response,

$$y = C + \left[\frac{D - C}{1 + \left(\frac{X}{I_{50}} \right)^b} \right] \qquad (1)$$

where C=lower limit, D=upper limit, b=slope, and I_{50}=dose giving 50% response (for plant biomass growth response - GR_{50}) (Seefeldt et al., 1995).

[14]C-diclosulam Translocation in Bristly Starbur. Plants were grown in the same manner as previously described. Two days prior to experiment initiation, bristly starbur plants were moved from the greenhouse into a growth chamber with a constant 28 C temperature and 50% relative humidity when they were in the 4th to 6th leaf stage of growth. Growth chamber lighting was provided by fluorescent and incandescent lamps at 450 μE/m^2/s. The study was conducted as a randomized complete block design with treatments arranged as a split-plot and with 8 replicates. Whole plots were biotypes, and sub-plots were plant parts harvested. The study was conducted and then repeated twice. Since no differences were noted for the biotypes for the dose response experiments, plants from the two locations were combined for the ^{14}C-diclosulam studies.

Prior to radiolabeling, the second fully expanded bristly starbur leaf (Li et al., 2005) was covered with polyethylene film. Then the entire plant was over sprayed with diclosulam at 0.8 g/ha mixed with deionized water and included a NIS at 0.25% (v/v), with a spray solution pH of 4.9. The film was then removed and the leaf was spotted with the radiolabeled solution using a microapplicator (Burkard Manufacturing Co. Ltd., Woodcock Hill Industrial Estate, Rickmansworth, Hertfordshire WD3 IPJ, U.K.). The spotting solution was prepared by mixing 0.5 ml of the spray solution with ^{14}C-labeled diclosulam (100:20 v/v). Technical grade phenyl ring labeled-^{14}C-diclosulam with 2.59 kBq/mg specific activity and 98% radiochemical purity was used. Ten 1-μl droplets of ^{14}C-diclosulam were placed on the adaxial leaf surface approximately 2 mm away from the center vein, beginning at the base of the leaf and moving parallel to the center vein

resulting in two lines of 5 drops on either side. Total specific activity applied contained 1.6 kBq of radioactivity. Plants were returned to the growth chamber immediately after spotting.

Bristly starbur plants were then harvested at 24 and 48 hour after treatment. Research on flumetsulam and other herbicides from the same triazolopyrimidine sulfonanilide chemical family as diclosulam, indicated maximum absorption occurred 16 to 30 hours after treatment (Frear et al., 1993; Hodges et al., 1990). Plants were cut at the soil line and sectioned into four parts: treated leaf, tissue above the treated leaf, tissue below the treated leaf, and roots. Soil was removed by washing the roots over a wire grid. Treated leaves were rinsed twice for 15 seconds with 5 ml of methanol:deionized water (1:1, v:v) to remove non-absorbed ^{14}C-diclosulam (Li et al., 2005). A 1-ml aliquot of the each rinsate was added to 10 ml of scintillation fluid, and radioactivity was quantified by liquid scintillation spectrometry. All plant parts were dried for 48 hours at 45 C, weighed, and combusted with a biological sample oxidizer. Radioactivity in the oxidized samples was quantified by liquid scintillation spectrometry. The amount of herbicide absorbed was calculated as the total radioactivity recovered from oxidation of the four plant parts and expressed as a percent of the total radioactivity applied. Distribution of ^{14}C-diclosulam in various plant parts was expressed as the percentage of total absorbed radioactivity.

All data for ^{14}C-diclosulam experiments were subjected to ANOVA and tested for experiment interactions.

Results and Discussion

Dose Response. ANOVA indicated there was no treatment by bristly starbur population interactions; therefore data were combined across populations for regression using equation [1]. Diclosulam injury symptoms on bristly starbur included stunting, chlorosis, and necrosis 14 DAT, with some plant death by 21 DAT. The log-logistic dose response for diclosulam I_{50} for bristly starbur based on visual control ratings was 0.92 g a.i./ha (Figure 2). The diclosulam GR_{50} on a foliar tissue dry matter weight basis was 0.95 g a.i./ha (Figure 3). The registered field use rate for diclosulam POST is 27 g a.i./ha, indicating a susceptibility for bristly starbur of 29x and 28x for the I_{50} and GR_{50}, respectively. Diclosulam molecular weight is 406.22 g/mole (Senseman, 2007), which corresponds to 457 µM at 26 g a.i./ha and 140 L/ha spray volume. Based on the application rates and spray solution volume for the dose response
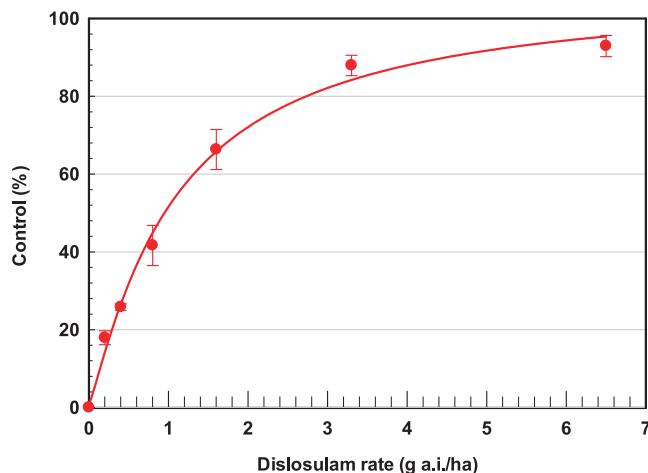

Figure 2. Log-logistic dose-response control curve and I_{50} value (number in parentheses represent the standard error) of bristly starbur (*Acanthospermum hispidum*) to diclosulam three weeks after application. The line represents the first-order regression equation and data points are the means of replications with bars indicating the standard error of the mean:

$$Y = 0.0 + \left(\frac{[100 - 0.0]}{1 + \left(\left[\frac{x}{0.916} \right]^{-2.93} \right)} \right) \qquad I_{50} = 0.92 \, (\pm 0.12) \text{ g a.i./ha}$$

experiments, concentrations of diclosulam were 3.5, 7.1, 14.2, 29.6, 57.1, and 114.3 µM for the 0.2 0.4, 0.8, 1.6, 3.3, and 6.5 g a.i./ha, respectively. These data indicate the extreme sensitivity of bristly starbur to diclosulam as compared to other weeds that require the full use rate for peanut weed control including
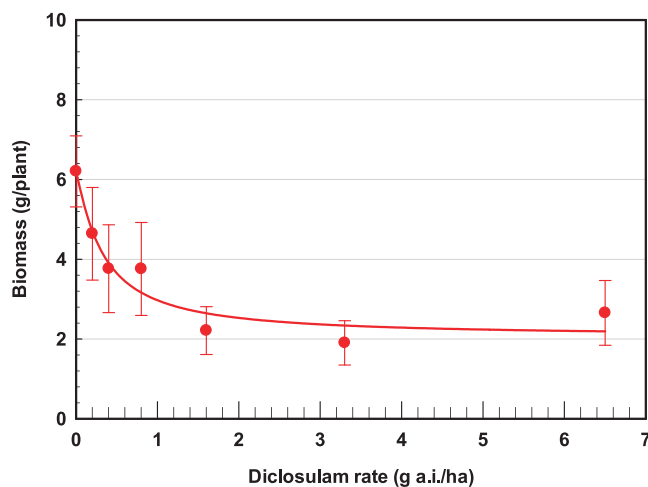

Figure 3. Log-logistic dose-response shoot dry weight curve and growth reduction GR_{50} value (number in parentheses represent the standard error) of bristly starbur (*Acanthospermum hispidum*) to diclosulam three weeks after application. The line represents the first-order regression equation and data points are the means of replications with bars indicating the standard error of the mean:

$$Y = 0.0 + \left(\frac{[6.52 - 0.0]}{1 + \left(\left[\frac{x}{0.95} \right]^{1.04} \right)} \right) \qquad GR_{50} = 0.95 \, (\pm 1.1) \text{ g a.i./ha}$$

Table 1. Plant biomass and distribution of ^{14}C-diclosulam over time in bristly starbur (*Acanthospermum hispidum*) as a percentage of material absorbed and Becquerel per mg of plant tissue.

Plant section[a]	Hours after treatment 24	48	Within time Pr > F[b]	Hours after treatment 24	48	Within time Pr > F	Hours after treatment 24	48	Within time Pr > F
	dry weight (mg)			%^{14}C-diclosulam absorbed			Bq ^{14}C-diclosulam/mg tissue		
Treated leaf	41.5	41.5	0.9890	31.8	35.1	0.4197	6.1	5.1	0.3934
Above	79.1	84.5	0.6181	58.1	55.3	0.6111	10.2	8.8	0.6296
Below	339.2	370.1	0.3945	8.8	7.8	0.6885	0.6	0.3	0.1270
Roots	264.5	264.2	0.9917	0.9	1.1	0.1035	0.06	0.06	0.9969
Total	724.3	760.3	0.5317	100	100	—	17.1	14.0	—

[a]Numbers indicate the percent distribution of ^{14}C-diclosulam among the four sectioned portions of each plant.
[b]ANOVA utilizing GLIMMIX procedure in SAS (SAS Institute, Cary, NC)

Benghal dayflower (Sabila *et al.*, 2012), smallflower morningglory [*Jacquemontia tamifolia* (L.) Griseb.], common cocklebur (*Xanthium strumarium* L.), prickly sida (*Sida spinosa* L.) and common ragweed (Everman *et al.*, 2006; Grey *et al.*, 2001). Tolerant crop and weed species have limited translocation and rapid metabolism of diclosulam (Senseman, 2007; Hodges *et al.*, 1990), which is similar to other sulfonamide herbicides (Frear *et al.*, 1993). Data presented by Hodges *et al.* (1990) noted GR$_{50}$ values of 0.8, 2.9, 2.7, and 2.1 g a.i./ ha for redroot pigweed (*Amaranthus retroflexus* L.), velvetleaf (*Abutilon theophrasti* Medik.), jimsonweed (*Datura stramonium* L.), and tall morningglory (*Ipomea purpurea* (L.) Roth], respectively based on spray applications of 190 L/ha. These data are similar to the GR$_{50}$ for bristly starbur in the current research of 0.33 g a.i./ha.

Based on these and previous data about the sensitivity of dicot species to diclosulam, it is suspected that bristly starbur does not readily metabolize diclosulam, resulting in low I$_{50}$/GR$_{50}$ values and extreme sensitivity (Figures 2 and 3). This level of diclosulam sensitivity in bristly starbur ((Prostko *et al.*, 2004) can be an issue as ALS herbicide resistance is exacerbated by extremely sensitive weed species. Multiple *Amaranthus* species (McNaughton *et al.*, 2005; Wise *et al.*, 2009), common cocklebur (Schmidt *et al.*, 2004), and common ragweed (Taylor *et al.*, 2002) have been reported to have resistance to POST applied ALS herbicides. Additionally, weeds that have exhibited ALS resistance have point mutations within their genes encoding for ALS. Specifically, formally susceptible biotypes have Ala 122, Pro 197, Ala 205, Tip 574, or Ser 653 point mutations that are replaced by various other amino acids that have conferred ALS resistance (Tranel and Wright, 2002). In an effort to hinder the development of herbicide resistance, it will be pertinent in peanut to maintain current weed control PRE, AE, and POST herbicide applications utilizing multiple mechanisms of action to prevent selection for ALS resistance in bristly starbur.

^{14}C-diclosulam Absorption and Translocation. There were no differences in plant biomass (mg per plant part and whole plant total) between the 24 and 48 hour after application timings (Table 1). Approximately 80% of the total applied radioactivity (1.28 of 1.6 kBq applied per plant) was recovered from leaf washes and oxidation of plant parts (n = 46 plants). Bristly starbur plants absorbed 65 and 55% of the applied ^{14}C-diclosulam at 24 and 48 hours after treatment, respectively (Data not shown). There was no explanation as to why 10% less ^{14}C-diclosulam was absorbed for the population of 48 hours plants verses the 24 hours treatments.

There were no differences in ^{14}C-diclosulam as a percentage of absorption and translocation for the treated leaf, foliage above the treated leaf, foliage below the treated leaf, or the roots for the 24 and 48 hour after application harvest timings (Table 1). ^{14}C-diclosulam moved acropetally out of the treated leaf 24 and 48 hr after treatment harvest (Table 1). Greater than 55% of the ^{14}C-diclosulam absorbed translocated to the shoot above the treated leaf. Diclosulam is a weak acid (pKa = 4.09) (Senseman, 2007), and was anionic in charge for this experiment as the pH of the spray solution was 4.9. Upon application to the bristly starbur leaf surface, the anionic molecule was absorbed across the leaf cuticle via increased lipophilicity due to the halogens F and Cl on opposite ends of the diclosulam molecule (Jeschke, 2009). Once the diclosulam molecule entered the bristly starbur leaf cell, it was phloem translocated to the apical growing points. The F and Cl moieties increase the lipophicitiy and provide superior metabolic stability after phloem translocation that improves herbicidal activity (Jeschke 2009). This can partially explain the low diclosulam GR$_{50}$ of 0.95 g a.i./ha for bristly starbur (Figure 3). When an herbicide has a K$_{ow}$ less than zero, they tend to be

phloem translocated when foliar applied (Bromilow *et al.*, 1990). The K_{ow} for diclosulam at pH 7 is -0.047 (Senseman 2007), and this is a common plant intercellular pH. The molecular structure of diclosulam promotes lipophilic absorption across the leaf cuticle and intercellular pH of 7 and greater results in a negative K_{ow} that increases phloem mobility. The data indicated that ^{14}C-diclosulam had portioning ratios for above the treated leaf foliar tissue to roots of 65:1, and the above treated leaf foliar tissue to below treated leaf foliar tissue ratio of 6.6:1, with little to no root accumulation 24 hours after treatment. Similar results were observed with the 48 hour after treatment harvest.

Conclusions

Bristly starbur exhibited extreme sensitivity to diclosulam and this efficacy was attributed to the lipophilic absorption and K_{ow} phloem translocation of the molecule. While extremely effective for bristly starbur control, diclosulam is an ALS herbicide and weeds have previously demonstrated the ability to rapidly develop resistance to this mechanism of action due to point mutations. Therefore, it is highly recommended that when diclosulam is POST applied for Benghal dayflower control in peanut as registered, it is used in combination with other herbicides with different mechanisms of action that provide Benghal dayflower control. As most growers use PRE, AE, and POST herbicides in their production practices with multiple mechanisms of action, this should limit the potential for ALS resistant bristly starbur to occur. However, growers should always be aware of how weed populations change in their fields and respond appropriately to weed escapes as a means to minimize the occurrence, survival, and spread of herbicide resistant weeds.

Acknowledgements

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