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Rice (*Oryza sativa*) response to drift rates of glyphosate

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Abstract: Greenhouse and field studies were conducted to investigate response of two rice varieties, Priscilla and Cocodrie, to sub-lethal rates of glyphosate in terms of injury, shikimate accumulation and yield. In the greenhouse, more shikimate accumulated in Cocodrie than Priscilla at comparable glyphosate rates applied to plants at the three-leaf stage. In field studies, glyphosate was applied to both varieties when they were 74-cm tall and in the internode separation growth stage. Visual injury, plant height, and leaf-tissue samples for shikimate analysis were collected at 3, 7, 14, 21 and 28 days after treatment (DAT). Rice yield was also determined. Noticeable visual injury and height reduction to both varieties was observed as early as 7 and 3 DAT in Cocodrie and Priscilla, respectively. Shikimate levels in leaves began to increase in both varieties by 3 DAT in a dose-dependent manner and reached a peak between 7 and 14 DAT. Elevated shikimate levels were still detectable by 28 DAT. Similar levels of shikimate accumulated in both varieties at comparable glyphosate rates. However, glyphosate treatment at comparable rates reduced rice yields more in Cocodrie than in Priscilla. The highest rate of glyphosate reduced yield in Cocodrie by 92% whereas there was only a 60% yield reduction in Priscilla. Shikimate levels in glyphosate-treated rice were strongly correlated to yield reductions across both varieties and appeared to be a better predictor of yield reduction than was visual injury. Visual injury coupled with measured shikimate levels can be used collaboratively to identify glyphosate exposure and estimate subsequent rice yield reductions.

Keywords: drift detection; glyphosate; rice; *Oryza sativa*; shikimate; visual injury

1 INTRODUCTION

1.1 Glyphosate use and drift

Glyphosate is used extensively worldwide for post-emergence control of annual and perennial broadleaf, grass and sedge weeds.[1–3] Glyphosate is often used in non-crop situations, orchards, vineyards and roadsides, as well as to control existing vegetation prior to planting in row-crop and drilled cropping systems, and in-season in glyphosate-resistant (GR) cropping systems.[4–6] It is estimated that 7405 metric tons of glyphosate were used worldwide in 1997,[2] and that amount has increased substantially with the advent of GR soybean, cotton and corn. Glyphosate can be applied by ground or aerial equipment, and is often applied throughout the year to control existing vegetation in many agricultural and industrial settings.

With increasing use of glyphosate worldwide, the opportunity for drift of glyphosate onto non-target areas also increases. It is estimated that varieties resistant to glyphosate are planted on 11, 76 and 85% of corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and cotton (*Gossypium hirsutum* L.) hectares in the USA, respectively.[7] However, glyphosate-resistant rice (*Oryza sativa* L.) varieties have not been commercialized. Rice in the southern USA is routinely drill- or water-seeded in April to early May, and is in the vegetative to early reproductive growth stage when GR cotton and soybean are typically treated with glyphosate. Drift events onto rice are an ever-increasing problem in the rice production areas of Arkansas, Louisiana, Mississippi and Missouri, which account for approximately 75% of the annual US rice crop hectareage. In 2000, 140 cases of glyphosate drift onto rice via ground or aerial application were reported in Mississippi (Mike Taggert, Mississippi Department of Agriculture, pers. comm., 2004).

Sub-lethal rates of glyphosate can cause severe injury to rice and significantly reduce grain yield.[8,9] Rice in the reproductive growth stages from internode

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elongation to booting is more susceptible to glyphosate than rice in vegetative growth stages.[8] Glyphosate at 140 g ha^{-1} can reduce yields up to 99% if applied to rice in the boot growth stage.[8] However, when rice is exposed to glyphosate at the boot growth stage there is little visual injury.[8] Thus, there is a need for a quantitative measurement, other than visual injury, to predict yield loss.

1.2 Plant response to glyphosate

Glyphosate inhibits 5-enolypyruvylshikimate-3-phosphate synthase (EPSPS) and stops biosynthesis of the aromatic amino acids tryptophan, tyrosine and phenylalanine.[10,11] Inhibition of EPSPS results in the accumulation of shikimate due to unregulated flow of carbon into the shikimate pathway.[12–14] High levels of shikimate accumulate in susceptible species such as corn, soybean, cotton, and wheat (*Triticum aestivum* L.) after treatment with sub-lethal rates of glyphosate.[11–14]

A relatively simple and rapid assay has been used successfully to measure increased shikimate levels in glyphosate-treated susceptible plants of corn and soybean.[13] To date, this assay has not been used either to determine shikimate levels in glyphosate-treated rice or to estimate potential yield losses. The objectives of this study were (1) to characterize physical, physiological and yield response of rice to sub-lethal rates of glyphosate in the greenhouse and field, and (2) to evaluate shikimate levels over time in glyphosate-treated rice with the rapid assay described above.

2 MATERIALS AND METHODS

2.1 Greenhouse study

Seeds of the rice varieties Cocodrie and Priscilla were planted 1.5 cm deep in 9-cm diameter pots containing a mixture of soil (Bosket sandy loam, fine-loamy, mixed thermic Molic Hapludalfs) and Jiffy Mix potting soil (1 + 1 by volume). Plants were grown in a greenhouse with 32/25 (±3) °C day/night temperature. Natural light was supplemented with light from sodium vapor lamps to provide a 14-h photoperiod. After emergence, plants were thinned to two plants per pot.

2.1.1 Injury and shikimate study

An isopropylamine salt of glyphosate (Roundup Custom®) was applied at 0, 26, 105 and 420 g AE ha^{-1} to 31- to 37-cm-tall plants in the three-leaf growth stage. A nonionic surfactant (Induce®, a mixture of alkylarylpolyoxyalkane ethers and free fatty acids) was added at 2.5 ml liter^{-1} to each glyphosate solution. Treatments were applied using an air-pressurized indoor spray chamber equipped with an 8002E flat-fan nozzle calibrated to deliver a spray volume of 190 liter ha^{-1} at 140 kPa. After spraying, plants were returned immediately to the greenhouse. Herbicide efficacy was assessed 14 days after treatment (DAT) by visually estimating injury on each plant per pot on a scale of 0 to 100%, with 0 indicating no visual injury and 100 indicating death. Visual estimates of injury were based on foliar chlorosis, necrosis and plant stunting. Injury ratings were averaged for the two plants per pot.

Sufficient numbers of each variety were treated with each glyphosate rate so that tissues samples could be collected from fresh plants at 3, 7, 14 and 28 DAT. The youngest fully expanded leaf of each plant was clipped at the leaf collar and ten leaf discs (4 mm diameter) were excised from each leaf using a modified cork borer equipped with a spring-loaded plunger. Five discs were excised from each side of the leaf midrib equidistant between the mid-rib and leaf edge and approximately half-way between the leaf-tip and collar. Each leaf was approximately 10 cm in length. Leaf-discs from each leaf were placed in a screw-top 7-ml plastic vial. Discs were frozen and kept at −20 °C until analysis.

Leaf-discs were maintained in the freezer for <1 month before measuring shikimate levels according to previously published procedures.[15,16] Vials were removed from the freezer and 1 ml of 0.25 M hydrochloric acid was added to each vial. Vials were mixed by vortexing for 3 s to ensure that discs were in solution. Leaf-discs were incubated at 22 °C in the hydrochloric acid solution for 1.5 h. A 25-µl aliquot of solution was placed in two separate adjacent wells of a 96-well microtitre plate containing 100 µl of 0.25% periodic acid/0.25% meta-periodate solution in each well. Plates were incubated at 22 °C for 60 min. A 100-µl aliquot of 0.6 M sodium hydroxide/0.22 M sodium sulfite solution was then added to each well of each plate, and the absorbance at 380 nm was measured using a BIO-TEK Synergy HT spectrophotometer.

A shikimate standard curve was developed by adding known amounts of shikimate to wells containing leaf discs not exposed to glyphosate, so that shikimate levels could be reported as µg shikimate cm^{-2} of leaf area. Plates were set up so that leaf-discs placed in each column were from a single replication of both varieties. Shikimate data are presented as shikimate amount in treated rice minus background amount in untreated rice for each replication of each variety. Treatments were replicated three times in a completely randomized designed experiment, and the experiment was repeated.

2.2 Field studies

A dose–response study was conducted in commercial rice fields at two sites in 2004. The fields were located in Holmes (Site 1) and Bolivar (Site 2) counties of Mississippi. Soil types were a Dundee silt loam (Fine–silty, mixed, active, thermic Typic Endoaqualfs) and a Sharkey silty clay (very-fine, smectitic, thermic Chromic Epiaquert) for Sites 1 and 2, respectively. The rice varieties Priscilla (Site 1) and Cocodrie (Site 2) were drilled in 19-cm-wide rows at

100 kg seed ha^{-1} in early April at Site 1 and early June at Site 2. Common cultural management practices were imposed at each site. An isopropylamine salt formulation of glyphosate (Roundup Custom®) was applied at 0, 13, 26, 52, 105, 210, and 420 g AE ha^{-1} to rice in the internode separation growth stage on 18 June at Site 1 and 3 August at Site 2. Rice was 74-cm tall at both sites at the time of glyphosate application. A nonionic surfactant was added at 2.5 ml $liter^{-1}$ rate to each glyphosate solution. Glyphosate was applied using a carbon-dioxide-pressurized backpack sprayer calibrated to deliver 140 liter ha^{-1} at 193 kPa. The Plot size was 3 m wide by 4.5 m long. Each treatment was replicated four times in a randomized complete block design experiment at both sites.

Visual injury was estimated 3, 7, 14, 21 and 28 DAT at the whole-plot level on a scale of 0 to 100%, with 0 indicating no visual injury and 100 indicating death. Visual estimates of injury were based on foliar chlorosis, necrosis and plant stunting. Plant height was measured at the same timings as visual injury by recording the height of ten randomly selected plants per plot. Plant height was determined by measuring the distance between ground level and the leaf-tip of the youngest leaf held parallel to a ruler. Height data are presented as percentage height reduction compared to height of untreated rice in each replication.

The largest tiller was clipped from five randomly selected plants per plot at the same timings as visual injury ratings. The youngest fully expanded leaf was clipped from each tiller at the leaf collar. Five leaf discs (4 mm diameter) were excised from each leaf using the same cork borer as in the shikimate greenhouse study described in Section 2.1.1. Discs were harvested equidistant between the mid-rib and leaf edge and approximately half-way between the leaf-tip and collar. Each leaf was approximately 20 cm in length. Leaf-discs were placed in a screw-top 7-ml plastic vial. Vials were placed on ice in a cooler and transported to the laboratory where they were stored for <3 months at −20 °C until analysis. Shikimate levels were measured using the same procedure as described in Section 2.1.1.

Rough rice grain yields were estimated by harvesting the centre 6.75 m^2 of each plot with an Almaco PMC 20 plot combine equipped with a HarvestMaster Harvest Data System which measured plot yield and grain moisture content. Plot yield data were standardized to 120 g kg^{-1} moisture for data analyses. A 125 g sample of rough rice was collected from each plot at harvest, and standard milling procedures were applied to these samples to determine the relative percentage of whole kernels.[17]

2.3 Data analysis

2.3.1 Greenhouse study

Injury and shikimate data were subjected to analysis of variance.[18] Main effects and interactions were tested by the appropriate mean square associated with the random variables. Data were pooled across experiment because of the lack of experiment interaction. Data were presented separately for each variety and sampling date because of a significant variety by sampling date interaction. Treatment means were separated at the 5% level of significance using Fisher's protected least significant difference test.

2.3.2 Field studies

Data across field sites had similar trends, but interactions occurred across sites. Thus, data are presented independently for each site. Visual injury, height reduction and shikimate data were treated using analysis of variance. Relationships between glyphosate rate, visual injury, shikimate levels and rice yield were best fitted to the sigmoidal logistic regression Eqn (1).

$$Y = \frac{a}{1 + (X/X_0)^b} \quad (1)$$

In this equation, a is the difference of the upper and lower response limits (asymptotes), X_0 is the level of the independent variable that results in a 50% reduction (IC_{50}) in the dependant variable, and b is the slope of the curve around X_0. Pseudo-R^2 values were calculated to assess the goodness of fit for the appropriate equation. The R^2 value was obtained by subtracting the ratio of the residual sum of squares to the corrected total sum of squares from one. The residual sum of squares was attributed to that variation not explained by the fitted line. The R^2 and residual mean squares were used to determine the goodness-of-fit to the regression model.

3 RESULTS AND DISCUSSION

3.1 Greenhouse study

Injury to both varieties increased significantly with increasing glyphosate rate. Injury to Cocodrie and Priscilla ranged from 10 to 20% and 45 to 70% at rates of 26 and 420 g ha^{-1}, respectively (data not shown). Stunting of plant growth and chlorotic discoloration of tissue characterized injury.

Shikimate accumulated in leaves of both varieties as early as 3 DAT at 420 g ha^{-1} glyphosate (Table 1). Shikimate levels in treated rice increased between 3 and 14 DAT, and then declined by 28 DAT. However,

Table 1. Effect of glyphosate rate on shikimate accumulation in Priscilla (PR) and Cocodrie (CO) rice at 3, 7, 14, and 28 days after treatment (DAT) in the greenhouse

Glyphosate rate	3 DAT		7 DAT		14 DAT		28 DAT	
(g AE ha^{-1})	PR	CO	PR	CO	PR	CO	PR	CO
	μg shikimate cm^{-2}							
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	1.8	4.6	1.4	1.5	0.0	1.0
105	0.6	0.3	3.9	6.4	2.8	4.6	0.1	2.6
420	3.4	5.8	6.6	13.4	11.1	14.1	1.8	5.9
LSD (P < 0.05)	1.0		1.8		2.2		2.2	

by 28 DAT, shikimate levels in rice treated with 420 g ha^{-1} were still higher than levels in non-treated rice. These findings are different from those reported in literature,[12–14] in which shikimate levels in soybean, corn, cotton and wheat were highest between 3 and 7 DAT, and then decreased to levels similar to those in untreated plants with increasing time after treatment. Moreover, in rice there was also a peak in shikimate levels for both varieties by 7 DAT, although there were still significantly higher shikimate levels in the rice treated with 420 g ha^{-1} at 28 DAT. It is interesting that there was a varietial response, with levels of shikimate being higher in Cocodrie than in Priscilla treated with 420 g ha^{-1} particularly at 21 and 28 DAT.

3.2 Field studies

3.2.1 Physical and physiological response of rice

No visual injury to rice was observed by 3 DAT at either site (data not shown). Similar trends in degree of visual rice injury were observed at 7 to 28 DAT at both locations (Table 2). Injury at both sites increased with time after treatment and reached a maximum at 28 DAT. There was more visual injury on Priscilla at Site 1 than on Cocodrie at Site 2 at all rates except that of 420 g ha^{-1} at 28 DAT.

The effect of glyphosate on plant height followed a similar pattern to visual injury. Glyphosate treatment inhibited height more on Priscilla at Site 1 at all rates when compared with Cocodrie at Site 2 (Table 3). However, the maximum height reduction was only 15%. These results are similar to those previous studies in which glyphosate applied at the panicle initiation or boot stage causes little reduction in plant height.[8,9]

Shikimate levels were significantly higher in glyphosate-treated rice than in untreated rice as early as 3 DAT at both sites (Table 4). Shikimate levels peaked in both varieties between 3 and 7 DAT, followed by a decline in levels by 28 DAT. However shikimate levels remained significantly higher in treated *versus* untreated rice at 28 DAT. These are the first published data on shikimate accumulation with respect to time after glyphosate treatment of rice. Increased shikimate levels were maintained well after the first sign of visual injury and may serve as a physiological parameter that can be used to identify a glyphosate drift event. Levels of shikimate were higher in the Cocodrie rice than in the Priscilla rice, even though lower visual injury in the field was observed on Cocodrie than in Priscilla.

Table 2. Visual injury to Priscilla (Site 1) and Cocodrie (Site 2) rice at 7, 14, 21, and 28 days after treatment (DAT)[a]

Glyphosate rate (g AE ha^{-1})	Priscilla (DAT) 7	14	21	28	Cocodrie (DAT) 7	14	21	28
	%							
0	0	0	0	0	0	0	0	0
13	0	1	5	10	0	0	0	1
26	1	4	10	18	1	1	1	3
53	4	8	15	20	1	1	1	3
105	9	19	25	30	1	1	8	11
210	13	31	40	45	1	6	11	13
420	15	34	45	50	14	16	29	45
LSD ($P < 0.05$)	4	8	5	6	4	6	4	9

[a] No visual injury was observed at 3 DAT.

Table 3. Height reduction of Priscilla (Site 1) and Cocodrie (Site 2) rice at 3, 7, 14, 21, and 28 days after treatments (DAT)

Glyphosate rate (g AE ha^{-1})	Priscilla (DAT) 3	7	14	21	28	Cocodrie (DAT) 3	7	14	21	28
	%									
0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	1	3	3
26	1	1	2	2	2	0	0	1	3	3
53	2	5	10	10	10	1	1	2	4	3
105	3	8	15	14	14	1	1	2	5	7
210	3	9	16	15	14	1	1	3	5	8
420	4	11	17	15	15	1	2	4	6	13
LSD ($P < 0.05$)	NS	6	5	5	5	NS	NS	NS	NS	4

NS = not significant.

Table 4. Effect of glyphosate rate on shikimate accumulation in Priscilla (Site 1) and Cocodrie (Site 2) rice at 3, 7, 14, 21, and 28 days after treatment (DAT)

Glyphosate rate (g AE ha^{-1})	Priscilla (DAT) 3	7	14	21	28	Cocodrie (DAT) 3	7	14	21	28
	μg shikimate cm^{-2} leaf area									
13	1.3	1.3	0.6	1.0	1.0	5.1	4.1	1.3	0.6	0.3
26	1.9	2.9	1.6	2.2	1.6	5.4	4.5	2.9	1.0	0.6
53	2.2	3.5	2.9	2.9	1.6	6.1	5.1	3.5	1.3	1.0
105	2.5	4.1	3.2	3.5	2.9	8.3	7.0	6.7	2.2	2.9
210	4.1	5.4	4.5	3.8	3.5	8.6	8.6	7.6	3.8	4.8
420	5.1	6.4	6.1	5.1	4.1	15.6	10.2	8.9	6.4	8.6
LSD ($P < 0.05$)	1.3	1.0	2.2	1.0	1.0	4.1	1.6	1.6	1.0	1.6

Rough rice yield for untreated rice at Sites 1 and 2 were 9020 and 6195 kg ha^{-1}, respectively. The difference in yield across sites may be attributed to seeding date. Optimum yields typically occur in Mississippi when rice is seeded from early April to early May. Depending on the environment, rice yields generally decline as seeding date is delayed after May 1

Similar trends were observed for both varieties with respect to reduction of rough rice yield with increasing glyphosate rate. Rough rice yield decreased with increasing rates of glyphosate (Fig. 1). However, Cocodrie rice yield was reduced more than Priscilla rice yield at comparable glyphosate rates (Fig. 1): the estimated IC_{50} of glyphosate on Cocodrie was 60 g ha^{-1} compared with 339 g ha^{-1} for Priscilla. The differences in the sensitivity of these two varieties to glyphosate may be related to the physiological state of the plants at the time of treatment. Both varieties were sprayed at internode elongation when the plants were 74 cm tall. However, Cocodrie was planted much

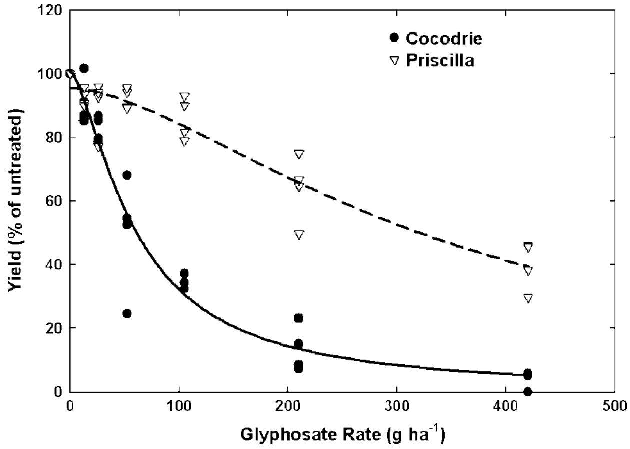

Figure 1. Effects of glyphosate rate on reduction of Priscilla (Site 1) and Cocodrie (Site 2) rough rice yield. Logistics equation for Priscilla: $Y = 95/1 + (X/339)^{1.7}$ ($R^2 = 0.99$). Logistics equation for Cocodrie: $Y = 100/1 + (X/60)^{1.5}$ ($R^2 = 0.99$).

later and may have been more physiologically mature at the time of spraying than Priscilla. Findings similar to these have been reported in other studies in which rice yield reductions of 0–65% have occurred when rice was treated with sub-lethal rates of glyphosate at the panicle-initiation growth stage.[8] Reduction in yield was characterized by fewer kernels, blanked kernels, deformation of panicle and parrot beaking of kernels.

No differences were detected in milling quality (data not shown). Theoretically, only parrot-beaked kernels would affect milling quality, because blanked or severely deformed kernels would be eliminated from the sample in the threshing process.

3.2.2 *Relationships between physical and physiological parameters*

There was a correlation between visual injury and shikimate content at both sites (Fig. 2). More shikimate accumulated in Cocodrie than in Priscilla at comparable injury levels. The cause of this difference is not known, although it could be due to the physiological state of the plants at the time of application or that Cocodrie was more sensitive to inhibition of EPSPS by glyphosate than Priscilla, resulting in greater accumulation of shikimate. The greenhouse study showed that more shikimate accumulated, and persisted for a longer period of time, in Cocodrie than in Priscilla at comparable glyphosate rates (Table 1). Because there is a good relationship between visual injury and increased shikimate level, these parameters may have the potential to be used together for detection of glyphosate drift. If at least 10% injury were observed, shikimate levels were significantly higher than levels in untreated rice at both sites.

The relationship between visual injury at 28 DAT and yield was consistent with previous research documenting poor correlation between visual injury and yield reduction in rice sprayed at the panicle initiation growth stage.[8] (Fig. 3). There was a much greater reduction in yield with Cocodrie than Priscilla at any given injury rating (Fig. 3). The estimated injury levels that correlated with a 50% reduction in yield for the two varieties were 10% and 53% for Cocodrie and Priscilla, respectively.

The relationship between shikimate levels and yield reduction varied depending on the DAT in which the analysis was conducted. Shikimate levels at 14 DAT were highly correlated to rice yield and there was little difference between Cocodrie and Priscilla (Fig. 4). However, shikimate levels at 21 and 28 DAT, although still highly correlated to yield, differed between the two rice varieties. There was a greater reduction in yield at the same shikimate levels in Cocodrie than in Priscilla. The differences were not as great as the differences between yield and visual injury for the two varieties.

Shikimate accumulation is an indicator of glyphosate inhibition of EPSPS and these results suggest that Cocodrie was more sensitive to glyphosate than was Priscilla. This apparent increased sensitivity of Cocodrie was manifested in greater yield reduction compared with Priscilla at the same glyphosate rate.

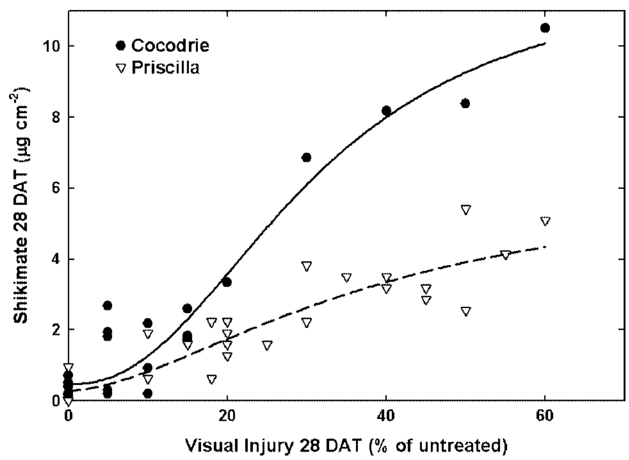

Figure 2. Relationship between visual injury 28 DAT and shikimate levels 28 DAT for Priscilla (Site 1) and Cocodrie (Site 2) rice. Logistics equation for Priscilla: $Y = 6/1 + (X/39)^{-1.7}$ ($R^2 = 0.93$). Logistics equation for Cocodrie: $Y = 12/1 + (X/31)^{-2.3}$($R^2 = 0.96$).

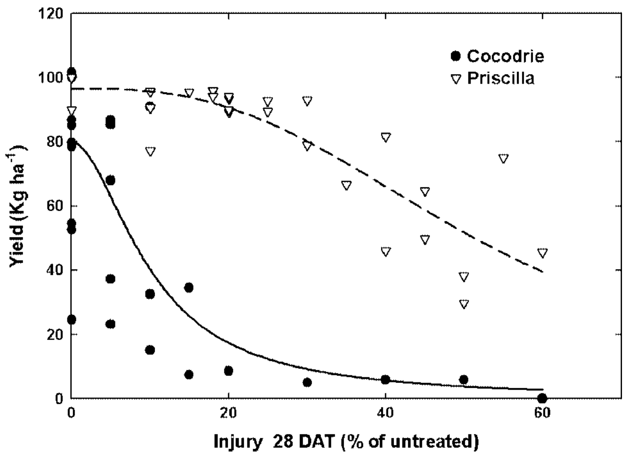

Figure 3. Relationship between visual injury 28 DAT and yield for Priscilla (Site 1) and Cocodrie (Site 2) rice. Logistics equation for Priscilla: $Y = 97/1 + (X/53)^{2.8}$ ($R^2 = 0.99$). Logistics equation for Cocodrie: $Y = 80/1 + (X/10)^{1.9}$ ($R^2 = 0.94$).

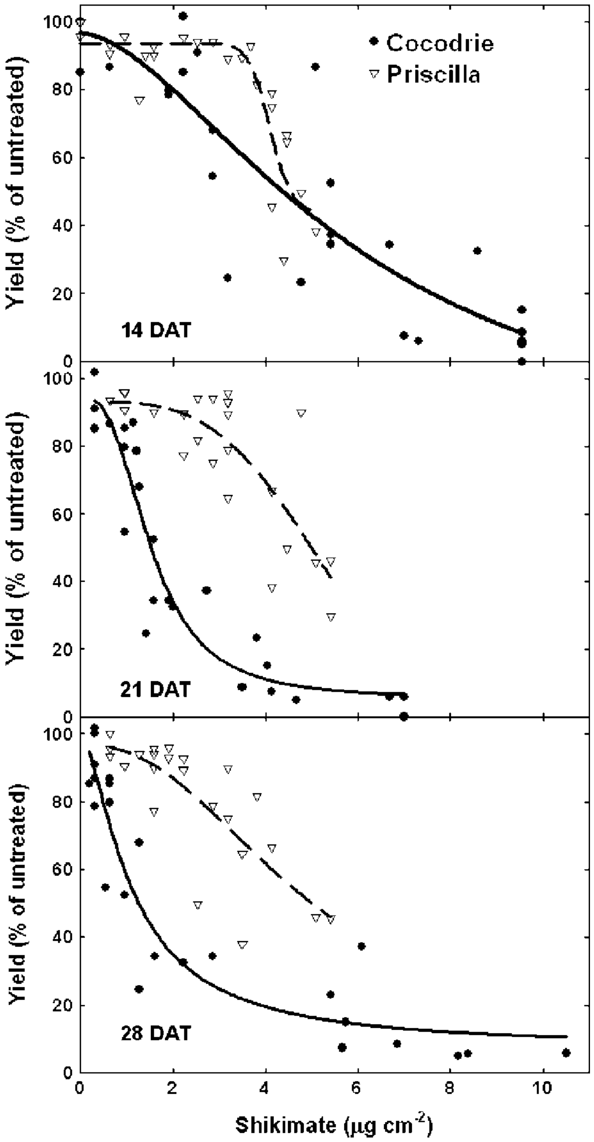

Figure 4. Relationship between shikimate levels at 14, 21 and 28 DAT and yield for Priscilla and Cocodrie rice. Logistics equations for Priscilla at 14, 21 and 28 DAT are: $Y = 95/1 + (X/4.7)^{7.5}$ ($R^2 = 0.99$); $Y = 93/1 + (X/5.2)^{4.1}$ ($R^2 = 0.98$); $Y = 92/1 + (X/2.3)^{5.2}$ ($R^2 = 0.98$), respectively. Logistics equations for Cocodrie at 14, 21 and 28 DAT are: $Y = 95/1 + (X/4.3)^{2.4}$ ($R^2 = 0.94$); $Y = 97/1 + (X/1.6)^{2.3}$ ($R^2 = 0.96$); $Y = 100/1 + (X/1.4)^{1.3}$ ($R^2 = 0.96$), respectively.

Shikimate levels may be a better indicator of potential yield reductions associated with a sub-lethal drift rate of glyphosate than visual injury.

4 CONCLUSIONS

Visual injury and yield response of both rice varieties to glyphosate was similar to those found previous research.[8,9] Visual injury was apparent by 7 DAT and was a better parameter than height reduction for confirming glyphosate exposure. Shikimate levels were at least $3\,\mu g\,cm^{-2}$ above levels in untreated rice when a noticeable degree of visual injury (>10%) was observed in the field. Shikimate levels remained elevated at the last sampling date of 28 DAT. Elevated shikimate levels in rice as late as 28 DAT have not been reported for other species, where shikimate levels often increased by 3–7 DAT and then returned to levels in untreated plants after 7 DAT.[12–15] Visual injury and measurement of shikimate levels have the potential to be used in combination for identifying glyphosate drift onto rice. Once visual injury is apparent, leaf-tissue samples can be analyzed for shikimate levels to determine whether a glyphosate drift event has occurred.

Significantly higher shikimate levels in leaves of glyphosate-treated plants can be detected well after the first sign of visual injury. Thus, it may not be so critical that leaf tissue be collected within a few days of glyphosate application from suspected rice plants as for other species where shikimate levels often return to levels in untreated plants soon after treatment. Because shikimate levels were higher in glyphosate-treated *versus* healthy rice for up to 28 DAT, the rapid spectrophotometric leaf-disc assay can be used to confirm glyphosate exposure well after the exposure event occurred. Measuring increased shikimate levels with the leaf-disc assay will also help to rule out drift of herbicides other than glyphosate, as glyphosate is the only herbicide that inhibits the EPSPS pathway.[11] This research demonstrates that a drift event can be detected and any subsequent effect on rice yield can be measured, especially if the rice is exposed to sub-lethal rates of glyphosate at the beginning of the reproductive growth stage. The differences observed across sites warrant further investigation. Varietial sensitivity or environmental conditions at time of glyphosate application may have affected rice response.

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