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Response of Giant Reed (*Arundo donax*) to Asulam and Trifloxysulfuron

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Giant reed has been proposed as a bioenergy crop in the sugarcane production region of south Florida, where it has a high invasive potential. In an effort to limit future invasion of giant reed escapes in sugarcane, currently labeled sugarcane herbicides asulam and trifloxysulfuron were evaluated for its management. Greenhouse and field dose–response studies were conducted at the Everglades Research and Education Center in Belle Glade, FL, between 2010 and 2011. Herbicides were applied at rates ranging from 0.46 to 7.4 kg ha^{-1} asulam and 2 to 32 g ha^{-1} trifloxysulfuron, which represent 0.125× to 2× sugarcane labeled use rates, respectively. In the greenhouse, asulam and trifloxysulfuron reduced giant reed relative shoot dry weight by a maximum of 50% at 21 d after treatment (DAT). The probability of giant reed resprouting 35 d following herbicide treatment was greater for trifloxysulfuron when compared with asulam. In the field, it was predicted that a maximum of 69 and 55% giant reed control occurred with application of asulam and trifloxysulfuron, respectively, at 14 DAT. Relative shoot dry weight of giant reed treated with asulam and trifloxysulfuron was reduced by a maximum of 43% at 42 DAT. Application of asulam and trifloxysulfuron did not provide complete control of giant reed at twice the labeled sugarcane use rate, indicating that control of established giant reed in sugarcane with currently available herbicides would not be an option.
Nomenclature: Asulam; trifloxysulfuron; giant reed, *Arundo donax* L.; sugarcane, *Saccharum* spp. hybrids.
Key words: Herbicide, postemergence, control, relative shoot dry weight, invasive.

Arundo donax ha sido propuesto como un cultivo bio-energético en la región de producción de caña de azúcar al sur de la Florida, donde tiene un alto potencial invasivo. En un esfuerzo por limitar alguna invasión futura de *A. donax* en la caña de azúcar, se evaluó su manejo con asulam y trifloxysulfuron, los cuales son herbicidas recomendados para este cultivo. Entre 2010 y 2011, se llevaron a cabo estudios de respuesta a dosis en invernadero y campo en el Everglades Research and Education Center en Belle Glade, FL. Los herbicidas fueron aplicados a dosis que variaron de 0.46 a 7.4 kg ha^{-1} de asulam y de 2 a 32 g ha^{-1} de trifloxysulfuron, que representan, respectivamente, 0.125× a 2× de las dosis recomendadas para caña de azúcar. En invernadero, asulam y trifloxysulfuron redujeron el peso seco relativo de la parte aérea de *A. donax* en un máximo de 50% a los 21 días después del tratamiento (DAT). La probabilidad de que *A. donax* rebrote 35 días después del tratamiento con el herbicida fue mayor para trifloxysulfuron cuando se comparó con asulam. En el campo se predijo que un máximo de 69 a 55% de control de *A. donax* ocurriría con la aplicación de asulam y trifloxysulfuron, respectivamente, a los 14 DAT. El peso seco relativo de la parte aérea de plantas de *A. donax* tratadas con asulam y trifloxysulfuron se redujo en un máximo de 43% a los 42 DAT. La aplicación de asulam y trifloxysulfuron no proporcionó un control completo de *A. donax* al doble de la dosis recomendada para la caña de azúcar, lo que indica que el control de *A. donax* establecido en caña de azúcar con los herbicidas actualmente disponibles, no sería una opción.

Growing energy demands and a desire to reduce carbon dioxide emission from fossil-based energy sources have given impetus for research in new crops as sources of biomass for energy production. Several perennial grasses that produce lignocellulosic biomass, including giant reed, have been evaluated as candidate crops. Giant reed has been proposed as potential feedstock for bioenergy production in the sugarcane (*Saccharum* spp. hybrids) production region of south Florida. Giant reed is a perennial clump forming rhizomatous C_3 grass native to East Asia and the Mediterranean region (Dudley et al. 2008; Lewandowski et al. 2003). It forms extensive monospecific stands that aggressively exclude competitors for light, nutrients, and water (Davis et al. 2010; Lambert et al. 2010) and spreads primarily through rhizome elongation and fragmentation (Khudamrongsawat et al. 2004) and not by seed, despite having a large inflorescence (Johnson et al. 2006). Giant reed exhibits traits ideal for bioenergy crops, including rapid growth, high productivity, low input requirements, and resistance to biotic and abiotic stresses (Heaton et al. 2004; Mariani et al. 2010). The high productivity of giant reed is attributed to its ability to capture and convert available solar energy into harvestable biomass with a maximal efficiency similar to C_4 species, despite having a C_3 photosynthetic cycle (Rossa et al. 1998). Angelini et al. (2009) reported a favorable net energy yield of 637 GJ ha^{-1} and biomass productivity of up to 37.7 T ha^{-1} of giant reed.

However, traits deemed ideal for bioenergy crops typify many of the traits commonly associated with invasive plants (Raghu et al. 2006). Giant reed has been reported as having a high invasive potential in Florida where cultivation is proposed (Barney and DiTomaso 2008; Gordon et al. 2011) using a modified Australian Weed Risk Assessment (Pheloung et al. 1999). The high invasive potential of giant reed in Florida is based on the widespread distribution of its propagules and inherent weedy characteristics, which greatly increases the likelihood of its escape and subsequent environmental damage (Barney and DiTomaso 2008). In addition, giant reed is nonnative to south Florida, thus compounding the potential risk of future invasions. Mack (2008) illustrated the difficulty of long-term containment of

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aggressively spreading escapes of nonnative species that became invasive.

To curtail future invasion of giant reed escapes if introduced as a bioenergy crop in sugarcane, there is need to screen available foliar-applied POST sugarcane grass herbicides for its management. Asulam (Asulox®, United Phosphorus, Inc., King of Prussia, PA) and trifloxysulfuron (Envoke®, Syngenta Crop Protection, LLC, Greensboro, NC) herbicides are labeled for POST control of annual and perennial grasses in sugarcane. Asulam is a carbamate herbicide that inhibits dihydropteroate (DHP) synthase, an enzyme involved in folic acid biosynthesis, resulting in inhibition of proteins and amino acids (Stephen et al. 1980; Veerasekaran et al. 1981a,b). Trifloxysulfuron is a sulfonylurea herbicide that inhibits acetolactate synthase, an enzyme involved in the biosynthesis of three essential branched-chain amino acids (LaRossa and Schloss 1984; Ray 1984). The labeled use rate for asulam in sugarcane is 2.8 to 3.7 kg ha^{-1} (Anonymous 2011a); for trifloxysulfuron, it is 16 g ha^{-1} (Anonymous 2011b). Over-the-top or semi-directed POST applications of these herbicides alone or in combination have been reported to provide control of rhizomatous perennial grasses, including torpedograss (*Panicum repens* L.) and johnsongrass (*Sorghum halepense* L. Pers.) in sugarcane (Dalley and Richard 2008; Hossain et al. 2001; Millhollon 1976). However, it is unclear what the response of giant reed would be to each of these herbicides. Consequently, the objective of this study was to compare response of giant reed to POST over-the-top application of asulam and trifloxysulfuron.

Materials and Methods

Greenhouse study. A greenhouse study was conducted at the University of Florida Everglades Research and Education Center (EREC) in Belle Glade, FL, to evaluate the response of giant reed to asulam and trifloxysulfuron. Giant reed stem cuttings 10-cm long with a bud were directly planted (two cuttings per pot) in 25-cm-diam pots filled with 1 : 1 Dania muck soil to potting soil mixture (Fafard Mixes for Professional Use, Conrad Fafard, Inc., Agawan, MA). Pots were thinned to one plant per pot 14 d after emergence. The experimental design was a completely randomized design with a two-factor factorial arrangement and four replications. The first factor was herbicide (asulam or trifloxysulfuron), and the second factor was herbicide rate (i.e., 0, 0.125×, 0.25×, 0.5×, 1×, and 2× labeled use rates. Application rates were 0, 0.46, 0.92, 1.85, 3.7, and 7.4 kg ha^{-1} of asulam and 0, 2, 4, 8, 16, and 32 g ha^{-1} of trifloxysulfuron. All herbicide treatments were applied with a non-ionic surfactant (Preference®, Winfield Solutions, LLC., St. Paul, MN) at 0.25% (v/v). Herbicide treatments were applied broadcast with a CO_2-pressurized backpack sprayer calibrated to deliver 180 L ha^{-1} of total volume at a pressure of 276 kPa. Giant reed plants were 4 wk old and 10 to 12 cm in height at the time of treatment. Plants were harvested at soil level 21 d after treatment (DAT) and dried in an oven for 48 h at 80 C to determine aboveground shoot dry weight. Shoot dry weight was divided by the mean shoot dry weight of the nontreated control to obtain the relative shoot dry weight (expressed as a percentage of the nontreated control). The binomial response of presence or absence of new giant reed resprouts were recorded as 1 or 0, respectively, 14 d after aboveground biomass harvesting (equivalent to 35 DAT) to determine the probability of giant reed resprouting after herbicide treatment. Two experimental runs were conducted for the study. The first run was planted October 7, 2010, and the second run was planted November 10, 2010.

Analysis of variance was conducted on relative shoot dry weight to determine the effect of herbicide, rate, and their interactions using the lme function in R (Pinheiro and Bates 2000). Nonlinear regression analysis was then performed on the relative shoot dry weight data using the drc package of R (R Development Core Team 2009; Ritz and Streibig 2005). A four-parameter log-logistic model (Seefeldt et al. 1995) was selected after inspection of several models:

$$f(x) = c + (d - c)/1 + \exp\{b[\log(x) - \log(e)]\} \qquad [1]$$

where $f(x)$ is relative shoot dry weight, x is the herbicide rate, b is the relative slope at the inflection point, c is the lower limit, d is the upper limit, and e is the inflection point of the fitted line (equivalent to the dose required to cause 50% response [ED_{50}]).

For the resprouting data, a generalized linear model was used to conduct analysis of deviance using the glm function in R (Venables and Ripley 2002). This analysis is analogous to ANOVA, in that it allows testing the data for significant effects of herbicide type and rate but is appropriate for the binomial nature of the resprouting data. After analysis of deviance, a two-parameter log-logistic model (Equation 2) was fit to the resprouting data using the drc package in R to determine the probability of resprouting after herbicide treatment. The two-parameter log-logistic model is similar to Equation 1, but the upper and lower limits are constrained to 1 and 0, respectively,

$$f(x) = 1/1 + \exp\{b[\log(x) - e]\} \qquad [2]$$

where $f(x)$ is the probability of giant reed resprouting, and x, b, and e are the same as in Equation 1. The ED_{50} of each herbicide type was compared using likelihood ratio tests when the effect of herbicide type was significant.

Field study. Field studies were conducted at two sites in 2011 at the University of Florida EREC in Belle Glade, FL. These two sites were bioenergy demonstration fields at EREC with an established giant reed population planted in 2007 on Dania muck soil (Euic, hyperthermic, shallow Lithic Haplosaprists) with pH 7.5 and 75% organic matter. This was the only established population of giant reed in the sugarcane production region of south Florida. Giant reed was harvested from these fields on March 10, 2011, before the establishment of the study after giant reed resprouting. The experimental design was a randomized complete block with a two-factor factorial arrangement and three replications. The first factor was herbicide (asulam or trifloxysulfuron), and the second factor was herbicide rate (i.e., 0, 0.25×, 0.5×, 1×, and 2× labeled use rates. Application rates were 0, 0.92, 1.85, 3.7, and 7.4 kg ha^{-1} of asulam, and 0, 4, 8, 16, and 32 g ha^{-1} of trifloxysulfuron. All herbicide treatments were applied with

Table 1. Model parameters and standard errors in parenthesis for the four- and two-parameter log logistic models (provided in Equations 1 and 2, respectively) for Figures 1 and 2 for giant reed response to asulam and trifloxysulfuron in greenhouse and field studies.

	Model parameter			
	b	*c*	*d*	*e*
Relative shoot dry weight at 21 DAT[a] in the greenhouse (Figures 1A and 2A)[b]	3.1 (1.28)	50.4 (3.78)	99.8 (4.43)	0.2 (0.03)
Relative shoot dry weight at 42 DAT in the field (Figures 1A and 2A)[c]	2.3 (2.42)	43.2 (22.63)	107.9 (10.51)	0.7 (0.27)
Control at 14 DAT in the field (Figure 1B)[d]	−2.6 (0.41)	0.3 (1.83)	68.5 (3.81)	0.7 (0.05)
Control at 14 DAT in the field (Figure 2B)[e]	−3.3 (0.65)	1.3 (1.79)	55.3 (3.27)	0.8 (0.06)
Probability of resprouting in the greenhouse (Figure 1C)[f]	3.0 (1.15)	—	—	1.3 (0.25)
Probability of resprouting in the greenhouse (Figure 2C)[g]	2.3 (1.40)	—	—	2.8 (1.37)

[a] Abbreviation: DAT, days after treatment.

[b] Combined response of giant reed to asulam and trifloxysulfuron in the greenhouse study at 21 DAT.

[c] Combined response of giant reed to asulam and trifloxysulfuron in the field study at 42 DAT.

[d] Giant reed control 14 DAT in response to asulam in the field study.

[e] Giant reed control 14 DAT in response to trifloxysulfuron in the field study.

[f] Probability of giant reed resprouting 14 d after aboveground biomass harvesting (equivalent to 35 d after asulam treatment) in the greenhouse study.

[g] Probability of giant reed resprouting 14 d after aboveground biomass harvesting (equivalent to 35 d after trifloxysulfuron treatment) in the greenhouse study.

a non-ionic surfactant at 0.25% (v/v). Experimental plots were 0.9 m wide by 5 m long. Herbicide treatments were applied in a manner similar to the greenhouse study to giant reed resprouts 15 to 20 cm in height on April 4 and 5, 2011, at the first and second site, respectively. A visual estimation of control was made with a scale of 0 (no control) to 100 (complete control) throughout the plot at 14 DAT. Aboveground plant biomass was harvested from an area 0.25 m^2 per plot at 42 DAT to obtain shoot dry weight and relative shoot dry weight using the procedure described in the greenhouse study.

ANOVA was conducted on control and relative shoot dry weight to determine the effect of herbicide, rate, and their interactions using the lme function in R (Pinheiro and Bates 2000). A four-parameter log-logistic model (Equation 1) was fit to control and relative shoot dry weight using the drc package of R, where $f(x)$, the response of interest, was either control or relative shoot dry weight.

Results and Discussion

Greenhouse study. No significant interactions with experimental run were detected, so data were combined for analysis. A significant effect of herbicide rate with respect to giant reed relative shoot dry weight at 21 DAT was observed ($P < 0.001$). Herbicide type and the interaction between herbicide type and rate were not significant, showing that giant reed responded similarly to both herbicides relative to their field use rates. The four-parameter log logistic model (Equation 1) provided the best fit to estimate the response of giant reed to POST application of asulam and trifloxysulfuron. A test for lack of fit at the 95% level was not significant for the curve, indicating that the regression model provided an appropriate fit to data (Ritz and Streibig 2005). Model parameters and their standard errors are provided in Table 1. Giant reed relative shoot dry weight decreased as asulam (Figure 1A) and trifloxysulfuron (Figure 2A) rates increased at 21 DAT. The response was similar for both asulam and trifloxysulfuron. On the basis of regression analysis, the ED_{50} for relative shoot dry weight was predicted to be 0.2× sugarcane use rate, which is equivalent to 0.89 kg ha^{-1}, and 4 g ha^{-1} for asulam and trifloxysulfuron, respectively. However, the mean response at high herbicide rates represented by the *c* parameter indicated that asulam and trifloxysulfuron reduced giant reed relative shoot dry weight by a maximum of 50%.

Significant effects of herbicide rate and type with respect to giant reed resprouting 14 d after aboveground biomass harvesting was observed. The two-parameter log logistic model (Equation 2) provided the best fit to estimate the probability of giant reed resprouting after POST application of asulam and trifloxysulfuron. A test for lack of fit at the 95% level was not significant for the curve, indicating that the regression model provided an appropriate fit to data (Ritz and Streibig 2005). Model parameters and their standard errors are provided in Table 1. The probability of giant reed resprouting deceased as rates of asulam (Figure 1C) and trifloxysulfuron (Figure 2C) increased. Trifloxysulfuron had a significantly higher ED_{50} with respect to the probability of resprouting of giant reed 14 d after aboveground biomass harvesting ($P = 0.030$). A threefold difference of the ED_{50} curves between trifloxysulfuron and asulam with respect to resprouting was observed, indicating that these herbicides differ in their ability to prevent resprouting of giant reed. This difference in resprouting might indicate that translocation to belowground biomass is less with trifloxysulfuron than with asulam, even though both herbicides had the same effect on aboveground biomass. McElroy et al. (2004) reported minor translocation of foliar-applied ^{14}C-trifloxysulfuron to the roots and newly formed rhizomes of green kyllinga (*Kyllinga gracillima* L.) and false-green kyllinga (*K. brevifolia* Rottb.). Similarly, Troxler et al. (2003) reported that neither yellow nutsedge (*Cyperus rotundus* L.) nor purple nutsedge (*Cyperus esculentus* L.) translocated more than 4% of foliar-applied ^{14}C-trifloxysulfuron to the tubers and roots. Additionally, most foliar applied ^{14}C-trifloxysulfuron has been reported not to translocate out of the treated leaves of many broadleaf plant species (Askew and Wilcut 2002; Wilcut et al. 1989). These studies indicate that trifloxysulfuron translocation to belowground biomass, including roots, is limited and probably explains the resprouting of giant reed observed in the present study. In contrast, Sharma et al. (1978) reported that

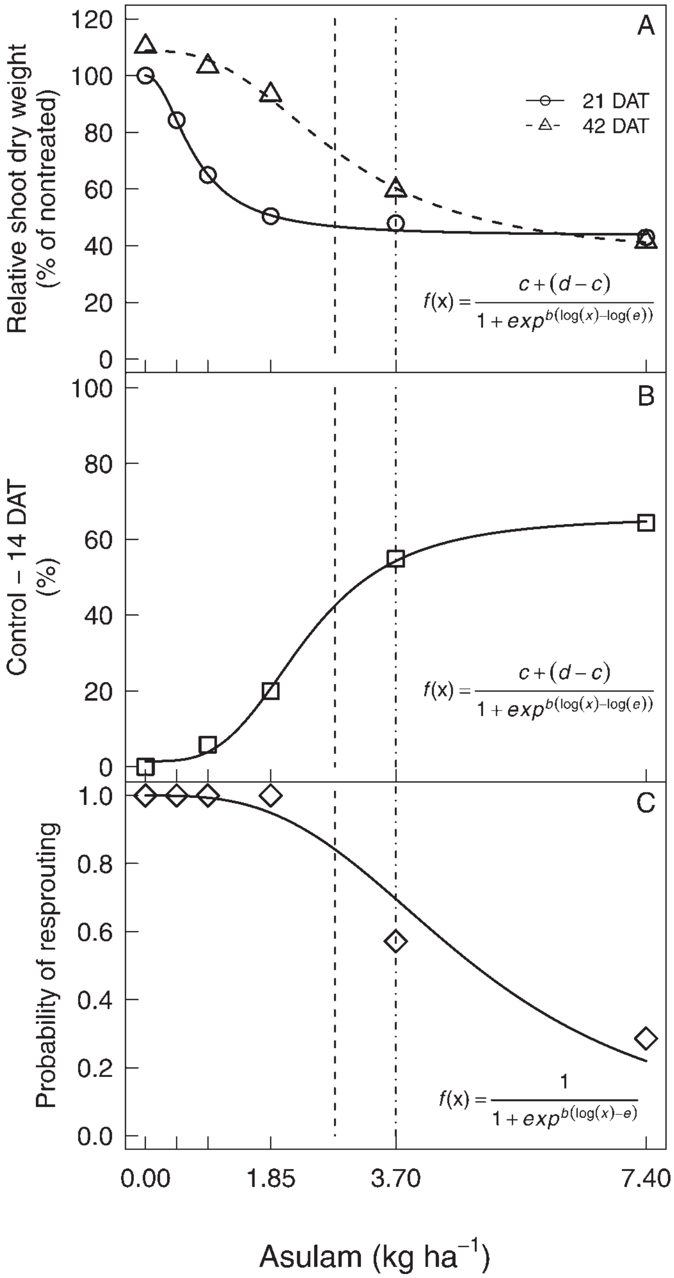

Figure 1. (A) Relative shoot dry weight of giant reed in response to asulam at 21 and 42 d after treatment (DAT) in greenhouse and field studies, respectively. (B) Percent control estimate of giant reed in response to asulam at 14 DAT in the field study. (C) Probability of giant reed resprouting 14 d after aboveground biomass harvesting (equivalent to 35 d after asulam treatment) in the greenhouse study. Labeled asulam sugarcane use rate of 2.8 (---) and 3.7 (-·-·) kg ha^{-1}, respectively. Model parameters are reported in Table 1.

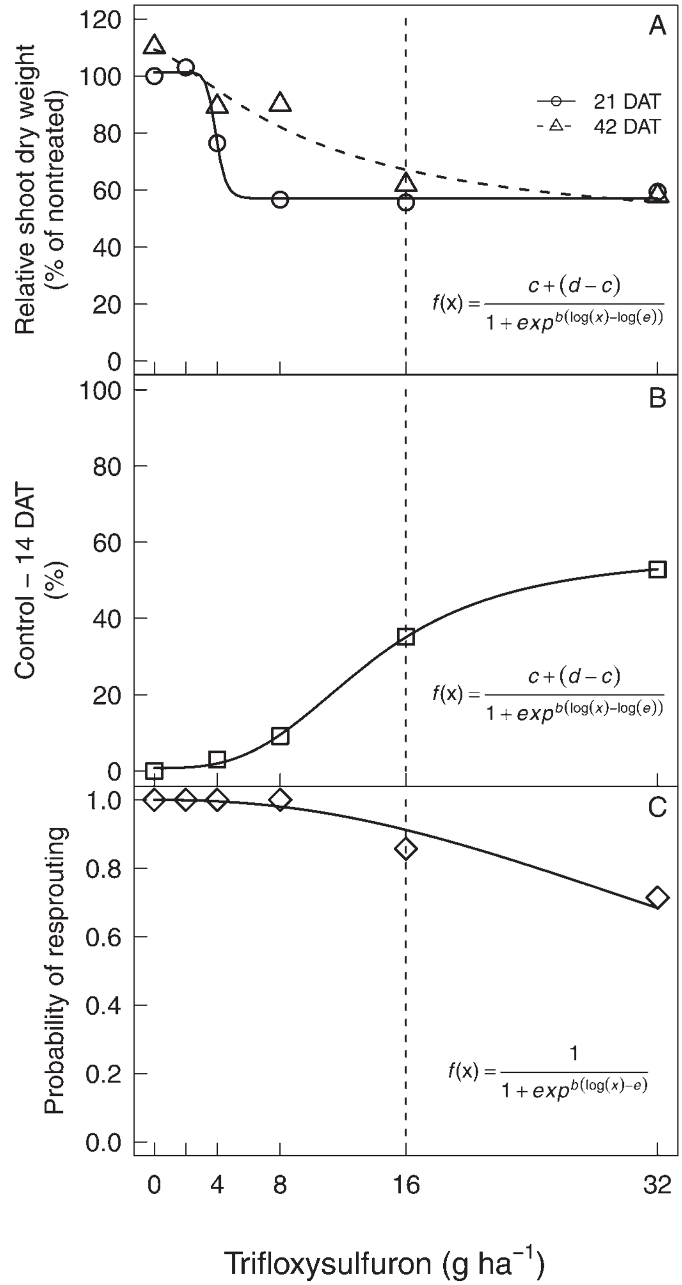

Figure 2. (A) Relative shoot dry weight of giant reed in response to trifloxysulfuron at 21 and 42 d after treatment (DAT) in greenhouse and field studies, respectively. (B) Percent control estimate of giant reed in response to trifloxysulfuron at 14 DAT in the field study. (C) Probability of giant reed resprouting 14 d after aboveground biomass harvesting (equivalent to 35 d after trifloxysulfuron treatment) in the greenhouse study. Labeled trifloxysulfuron sugarcane use rate of 16 ( ) g ha^{-1}. Model parameters are reported in Table 1.

^{14}C-asulam was distributed throughout wild oat (*Avena fatua* L.) plants after foliar application. Similarly, Veerasekaran et al. (1977) reported that asulam accumulated in the rhizomes of bracken fern (*Pteridium aquilinum* L. Kuhn) following foliar application. These studies indicate that asulam readily translocated to belowground biomass and probably explains the observed reduction in the probability of giant reed resprouting at the higher herbicide rates.

Field study. No significant interactions with experimental sites were observed, so data were combined for analysis. Significant effects of herbicide rate and type with respect to giant reed injury at 14 DAT ($P < 0.001$) were observed. The effect of herbicide rate was significant with respect to relative shoot dry weight at 42 DAT ($P < 0.001$), but the effect of herbicide type was not significant, indicating that at the labeled use rates, the two herbicides have a similar effect

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on giant reed shoot dry weight. Data were averaged over herbicide type for relative shoot dry weight for analysis because herbicide type and the interaction between herbicide rate and type were not significant. The four-parameter log-logistic model (Equation 1) provided the best fit to estimate the response of giant reed to POST application of asulam and trifloxysulfuron. A test for lack of fit at the 95% level was not significant for the control curve, indicating that the regression model provided an appropriate fit to data (Ritz and Streibig 2005). Model parameters and their standard errors are provided in Table 1. Giant reed control at 14 DAT increased as both rates of asulam (Figure 1B) and trifloxysulfuron (Figure 2B) increased. A onefold difference in control between asulam and trifloxysulfuron was observed with respect to ED_{50} at 14 DAT. However, the ratio of the ED_{50} curves was not significantly different from 1, indicating that giant reed response to asulam and trifloxysulfuron to cause a 50% response was not different. Regression analysis predicted a maximum of 69 and 53% giant reed control at 14 DAT with foliar application of asulam and trifloxysulfuron, respectively.

A test for lack of fit at the 95% level was not significant for the relative shoot dry weight curve, indicating that the regression model provided an appropriate fit to data (Ritz and Streibig 2005). Model parameters and their standard errors are provided in Table 1. Similar to the greenhouse study, relative shoot dry weight of giant reed decreased with increasing asulam (Figure 1A) and trifloxysulfuron (Figure 2A) rates at 42 DAT. The ED_{50} for relative shoot dry weight was 0.7× sugarcane use rate, equivalent to 2.59 kg ha^{-1} and 11 g ha^{-1} of asulam and trifloxysulfuron, respectively. The mean response of giant reed relative shoot dry weight at high herbicide rates indicated that asulam and trifloxysulfuron reduced giant reed relative shoot dry weight by a maximum of 43% at 42 DAT. Previous research has shown that asulam and trifloxysulfuron provides 73 and 42% control, respectively, of rhizomatous johnsongrass; however, combination of the two herbicides increased control up to 79% (Dalley and Richard 2001). Millhollon (1976) reported 51 and 61% control of johnsongrass by asulam. These results indicate that application of either asulam or trifloxysulfuron does not provide satisfactory control of giant reed at the labeled sugarcane use rate (1× rate equivalent to 2.8 to 3.7 kg ha^{-1} and 16 g ha^{-1} for asulam and trifloxysulfuron, respectively). Therefore, it is uncertain whether a tank mix of these herbicides can have an additive effect on giant reed similar to that observed on johnsongrass (Dalley and Richard 2001) on the basis of the low response of giant reed at the labeled use rates for sugarcane.

In greenhouse and field studies, asulam and trifloxysulfuron applied at the labeled sugarcane use rate of 2.8 to 3.7 kg ha^{-1} and 16 g ha^{-1}, respectively, did not provide complete control of giant reed. Asulam and trifloxysulfuron applied at twice the sugarcane labeled rate were also ineffective on giant reed control. Application of these herbicides at such rates would not be within the label use rate and could exacerbate sugarcane injury. Results show that containment of aggressively spreading giant reed in sugarcane would not be possible with the currently available herbicide control options.

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