

**Response of Asiatic dayflower (*Commelina communis* L.) to  
glyphosate and alternatives in soybean**

by

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*ABSTRACT*

Greenhouse experiments were conducted to evaluate Asiatic dayflower (*Commelina communis* L.) tolerance to glyphosate. Glyphosate rates and growth stages of Asiatic dayflower were evaluated. Shikimate accumulation in Asiatic dayflower, glyphosate resistant (GR) and non-GR maize and soybean cultivars was estimated. Field research was conducted to evaluate herbicides for Asiatic dayflower control. Pre-emergent and post-emergent herbicides were applied. Under greenhouse conditions, a single application of glyphosate ( $0.84 \text{ kg ae ha}^{-1}$ ) did not affect the growth of or shikimate accumulation in Asiatic dayflower. At an early growth stage (2 leaves),  $3.36 \text{ kg ae ha}^{-1}$  glyphosate provided 28% control. Susceptible maize and soybean and also Asiatic dayflower accumulated shikimate after glyphosate application. However, only Asiatic dayflower plants survived. In field experiments, metribuzin and KIH – 485 provided  $\geq 80\%$  and 73% control of Asiatic dayflower respectively. Early POST applications of cloransulam-methyl and lactofen provided 80 and 67% control of Asiatic dayflower respectively.

*RESUMEN (SPANISH)*

Se hicieron experimentos de invernadero para evaluar la tolerancia de la “flor del día asiática” (*Commelina communis* L.) a glifosato. Dosis de glifosato y estados de crecimiento de la maleza fueron evaluados. La acumulación de ácido shikímico fue evaluada en la “flor del día asiática” y en variedades de maíz y soya resistentes y no-resistentes a glifosato. Se hicieron experimentos de campo evaluando herbicidas para determinar cual es la mejor alternativa para controlar la “flor del día asiática” en cultivos de soya. Herbicidas pre-emergentes y post-emergentes fueron aplicados con las dosis más altas de acuerdo a las recomendaciones establecidas por los fabricantes. Bajo condiciones de invernadero, la aplicación de glifosato a la dosis recomendada,  $0.84 \text{ kg ae ha}^{-1}$  (1X), no afectó el crecimiento o la acumulación de ácido shikímico. Incluso aplicando el herbicida a plantas en estado de emergencia temprana (2 hojas) y usando una dosis alta (4X) glifosato solamente proporcionó un 28% de control. Plantas de maíz y soya susceptibles a glifosato acumularon ácido shikímico y murieron luego de 21 días de haber sido aplicadas. Sin embargo, las plantas de “flor de día asiática” sobrevivieron independientemente del la dosis de glifosato. Plantas de maíz y soya resistentes a glifosato no registraron ninguna acumulación de ácido shikímico. Bajo condiciones de campo los herbicidas pre-emergentes metribuzin y KIH – 485 proporcionaron 80 y 73% de control respectivamente. Los herbicidas post-emergentes cloransulam-methyl y lactofen proporcionaron un control de la “flor del día asiática” de 80 y 67 % respectivamente.

## CHAPTER 1: GENERAL INTRODUCTION

### Introduction

As hectares planted to glyphosate-resistant crops have increased in the past ten years, there is growing concern about the evolution of glyphosate-resistant weed species (Sprague 2002). The recurrent use of glyphosate has contributed to increased selection pressure and consequential development of resistance in several weed species (Koger et al. 2004). The exposure of weed communities to repeated glyphosate applications also caused weed population shifts in glyphosate-resistant crop fields. The issue of inconsistent weed control with glyphosate will continue to be an important topic into the future (Sprague 2002).

Glyphosate is an effective, broad-spectrum herbicide. The use of glyphosate for weed control includes the advantages of low cost and favorable toxicological and environmental profiles (Baylis 2000). The glyphosate mechanism of action is competitive inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [EC 2.5.1.19]. EPSPS catalyzes an essential step in the shikimic acid pathway, and glyphosate replaces the original substrate, phosphoenol-pyruvate, thus ultimately blocking the biosynthesis of important compounds such as the aromatic amino acids tryptophan, phenylalanine and tyrosine (Siehl 1997). Glyphosate is low in toxicity to mammals, birds, and fish, in part because EPSPS is present only in plants, bacteria and fungi (Padgett et al. 1996). Glyphosate readily binds to soil and is degraded by bacteria thus limiting the persistence in soil and potential groundwater contamination (Atkinson 1985).

The growing dependence on glyphosate for weed management in crops reinforces the importance of proper application timing specifically accounting for crop and weed growth stage (Waltz et al. 2004). Although glyphosate is a broad-spectrum herbicide, not all weeds are equally susceptible (Culpepper and York 2000; Payne and Oliver 2000). Inconsistent control of weeds can be attributed to reduced herbicide absorption, low herbicide translocation from the site of absorption to the site of action, metabolic detoxification of the herbicide, and altered target sites. Weeds treated with glyphosate can survive by using one or more of these mechanisms (Koger and Reddy 2005). Biotypes of goosegrass (*Eleusine indica*) in Malaysia, rigid ryegrass (*Lolium rigidum* Gaudin) in Australia, horseweed (*Conyza canadensis*) and common ragweed (*Ambrosia artemisiifolia*) in the United States, hairy fleabane (*Conyza bonariensis*) and buckhorn plantain (*Plantago lanceolata*) in South Africa and Italian ryegrass (*Lolium multiflorum* Lam.) in South America have evolved resistance to glyphosate (Lee and Ngim 2000; Pratley et al. 1999; VanGessel 2001; Perez and Kogan 2002; Heap 2006). Importantly, the number glyphosate-resistant species is increasing every year.

Herbicide resistance is described as the lack of response of a biotype in a weed population to an herbicide that develops over time (Stachler and Loux 2006). Resistance normally occurs after repeated use of the same herbicide or herbicides with the same site of action when some plants survive the application of the labeled recommended rate of the herbicide that usually provided satisfactory control. However certain weed species are inherently less affected by glyphosate. This



phenomenon is called herbicide tolerance, and is different than herbicide resistance because the occurrence is not the result of an evolutionary selection process.

Herbicide tolerance can be defined as the successful survival of most of the individual plants within a weed population; this could happen anytime that herbicide application occurs in the field (Paulson 2005; Roy 2004). DeGenaro and Weller (1984) reported a biotype of field bindweed (*Convolvulus arvensis* L.) to be naturally tolerant to glyphosate in a field with no history of glyphosate use. Variable response to glyphosate was also observed in tall morning-glory (*Ipomoea purpurea*), tropical spiderwort (*Commelina benghalensis*) and Asiatic dayflower (*Commelina communis* L.) (Paulson 2005; Jordan et al. 1997; Culpepper et al. 2004; Owen and Zelaya 2005). The weed species most likely to increase their presence in glyphosate-treated fields are those that have natural tolerance to glyphosate (Culpepper 2006). Generally, the lack of control for weeds treated with glyphosate in most of the cases is attributed to low application rates, large weed size, unfavorable environmental conditions, or a combination of these factors. However, the reason for poor control of the weeds described above did not appear to be the result of misapplication, rate selection, or environmental conditions.

Asiatic dayflower, an annual weed member of the Commelinaceae (spiderwort family) is indigenous to temperate north-east Asia, and frequently grows along crop fields, house yards and roads. Each plant has abundant inflorescences in which perfect flowers bloom before staminate flowers. Both perfect and staminate flowers open at sunrise and close about noon (Ushimaru 2003). Two varieties of

Asiatic dayflower are reported, *Commelina communis* var. *communis* and var. *ludens*. *Ludens* is distinguished by darker flowers and antherodes with maroon centers instead of entirely yellow (USDA, 2004). However, there is no research describing the behavior of the two varieties as weeds in soybean or maize fields.

Plants from the spiderwort family have become a control problem for farmers around the world. Asiatic dayflower has broad ecological tolerance that allows it to maintain successful ecological succession in a great variety of environments (Kutbay and Uckan 1998). Climbing dayflower (*Commelina diffusa*) is an alternative host of many tropical crop viral diseases (Baker and Zettler 1988). Tropical spiderwort has recently become the most troublesome weed in Georgia cotton production (Culpepper et al. 2004). Without the application of residual herbicides, *Commelina* sp. that germinate after a final application of glyphosate are still able to produce seeds and reduce harvest efficiency.

There are reports about ineffective control of *Commelina* sp. with glyphosate. Tropical spiderwort survived glyphosate applications in several crops fields in Brazil (Monquero et al. 2004). Researchers presumed that one of the tolerance mechanisms of tropical spiderwort to glyphosate could be differential penetration due to the chemical composition of the epicuticular wax, which contains lipophilic components in higher concentration than other studied species (Monquero et al. 2004). Other studies indicated that climbing dayflower recovered after glyphosate application presumably because of the high levels of starch in the leaves (Tuffi-Santos 2004).

Asiatic dayflower recently has become a problem for some producers in eastern Iowa. The anecdotal evidence of glyphosate tolerance and lengthy emergence period suggests that Asiatic dayflower will be difficult to manage in Roundup Ready<sup>®</sup> soybean and maize fields (Fawcett 2002). Pysek (2001) reported that Asiatic dayflower was one of the most aggressive weeds increasing its presence from 1972 to 1995 by 646% in the Czech Republic. Wiatrak et al. (2003) reported that control of Asiatic dayflower with glyphosate with 0.63 kg ae ha<sup>-1</sup> 10 and 21 days after treatment was 53 and 76%, respectively. Manabe et al. (1990) reported that glyphosate provided long term control of annual and perennial weeds except for Asiatic dayflower at four orchards in Japan. Tolerance of Asiatic dayflower seedlings to glyphosate was 49 times higher than that of glyphosate-susceptible maize seedlings. In the same experiment, the GR<sub>50</sub> of Asiatic dayflower seedlings to glyphosate was 6.8 times higher for the 5 leaf-stage plants than for the 3 leaf-stage (Park et al. 2004).

While literature exists that describes the response of Asiatic dayflower to a small number of herbicides and there have been botanical studies conducted with Asiatic dayflower; few studies have evaluated the response of Asiatic dayflower to glyphosate and other herbicides used in soybean and maize production. The present research evaluated the response of Asiatic dayflower to glyphosate and other herbicides used for weed control in soybean. The effects of herbicide rate and application timing on Asiatic dayflower were assessed under greenhouse and field

conditions. Additionally, shikimate accumulation in Asiatic dayflower, GR and non-GR maize and soybean was measured.

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## CHAPTER 2

**Response of Asiatic Dayflower (*Commelina communis*) to  
Glyphosate and Alternatives in Soybean**

A paper to be submitted to Weed Science

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## ABSTRACT

Asiatic dayflower (*Commelina communis* L.) has recently become a troublesome weed in eastern Iowa. This weed demonstrates an extended emergence period and there is anecdotal evidence of glyphosate tolerance. Thus, Asiatic dayflower is difficult to control in glyphosate-resistant (GR) maize and soybean. Greenhouse experiments were conducted to evaluate the response of Asiatic dayflower to glyphosate applied at different growth stages. Field research was conducted in 2005 and 2006 to evaluate different herbicides and determine the best tactic for Asiatic dayflower control in soybean. Pre-emergent herbicides (PRE) were applied at planting while post-emergent herbicides (POST) were applied separately 21 and 42 days after planting (DAP). In addition, shikimate accumulation in response to glyphosate applications was compared among Asiatic dayflower, GR and Non-GR maize and soybean.

Under greenhouse conditions, a single application of glyphosate (0.84 kg ae ha<sup>-1</sup>) did not control Asiatic dayflower. Only the highest rate evaluated, 13.44 kg ae ha<sup>-1</sup> (16X), was lethal to Asiatic dayflower. Even when applied at an early growth stage (2 leaves) and using high rates (3.36 kg ae ha<sup>-1</sup>), glyphosate provided just 28% Asiatic dayflower control.

In the field, metribuzin and KIH – 485 gave up to 80% and 73% Asiatic dayflower control respectively. Early POST applications (21 DAP) of cloransulam-methyl and lactofen provided 80 and 67 % control, respectively. A single glyphosate application at 0.86 kg ae ha<sup>-1</sup> provided approximately 50% Asiatic dayflower control.

Glyphosate-treated Asiatic dayflower, non-GR maize and soybeans accumulated shikimate after application. Twenty-one days after treatment, all the non-GR soybean and maize plants died; however, Asiatic dayflower plants survived. GR maize and soybeans did not accumulate shikimate in response to glyphosate.

## INTRODUCTION

Asiatic dayflower (*Commelina communis* L.), a monocot and a member of the spiderwort family (Commelinaceae), has started to become a serious problem for some soybean (*Glycine max* L.) farmers (Mishra 1997). Asiatic dayflower produces a great number of viable seeds and also can creep along the soil, rooting adventitiously at the nodes (Kuhns and Harpster 2005).

Asiatic dayflower is native to temperate north-east Asia (Ushimaru 2003); however, this weed is widely distributed around the world, principally in the northern hemisphere. In the United States, Asiatic dayflower is mostly located in the Midwest and the East coast (USDA 2004). Kutbay and Ucan (1998) reported that Asiatic dayflower shows broad ecological tolerance to climatic and soil factors; this phenotypic plasticity allows this specie to maintain dominance in several environments. Pysek (2001) observed that Asiatic dayflower is an aggressive introduced weed and expanded its distribution from 13 to 84 locations (646%) in the Czech Republic from 1972 to 1995.

Several members of the Commelinaceae have become difficult weeds to control for farmers around the world. Tropical spiderwort (*Commelina benghalensis* L.) has recently become the most troublesome weed in Georgia GR-cotton (*Gossypium hirsutum* L.) production (Culpepper et al. 2004). At this time, more than 80,000 ha are infested with tropical spiderwort and the number of hectares continues to increase (Culpepper et al. 2004; Webster et al. 2006). Interestingly, tropical

spiderwort has shown allelopathic behavior and reduced the germination and vigor of soybean and maize (*Zea mays* L.) seedlings (Singh et al. 1989). Asiatic dayflower has also been described as a difficult weed to control in Christmas tree plantations in Pennsylvania and Maryland (Kuhns and Harpster 2005).

Seed dormancy is an important characteristic of the spiderwort family. The longevity of Asiatic dayflower seeds in the soil is greater than other field weeds, and more than 80% of the seeds can germinate even after four and a half years in the soil seed bank (Takabayashi and Nakayama 1978). Additionally, plants from the Commelinaceae are known for their continuous germination throughout the growing season (Prostko et al. 2005; Fawcett 2002).

Glyphosate is a broad-spectrum herbicide registered for numerous crops and non-agricultural locations (Duke 1988). Glyphosate binds to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [EC 2.5.1.19] in the shikimic acid pathway, ultimately inhibiting the biosynthesis of the aromatic amino acids tryptophan, phenylalanine and tyrosine (Siehl 1997). Quantification of shikimate accumulation in response to glyphosate inhibition of EPSPS is a quick and accurate method to measure glyphosate-induced damage in sensitive plants (Mueller et al. 2003). As a result of the inhibition of the shikimic acid pathway, sensitive plants experience decreased synthesis of proteins, auxins, phytoalexins, folic acid, flavonoids, pathogen defense substances, and hundreds of other phenolic and alkaloid compounds (Bentley 1990; Siehl 1997).

The rapid adoption of GR crops such as maize, soybean, canola (*Brassica napus* L.) and cotton have allowed farmers to control grasses and broadleaf weeds throughout the growing season (Thomas et al. 2005). The number of hectares planted to GR crops has increased dramatically in the past 10 years, along with a growing concern about the evolution of glyphosate-resistant weed species (Sprague 2002). In recent years, the recurrent use of glyphosate has increased the selection pressure and hastened the consequential development of glyphosate resistance in several weed species (Koger et al. 2004). Biotypes of goosegrass (*Eleusine indica*) in Malaysia, rigid ryegrass (*Lolium rigidum* Gaudin) in Australia, horseweed (*Conyza canadensis*) and common ragweed (*Ambrosia artemisiifolia*) in the United States, hairy fleabane (*Conyza bonariensis*) and buckhorn plantain (*Plantago lanceolata*) in South Africa and Italian ryegrass (*Lolium multiflorum* Lam.) and Johnsongrass (*Sorghum halepense*) in South America have evolved resistance to glyphosate (Lee and Ngim 2000; Pratley et al. 1999; VanGessel 2001; Perez and Kogan 2002; Heap 2006). Based on these reports, it is apparent that the issue of glyphosate-resistant weeds will continue to be an important topic for years to come (Sprague, 2002).

Nevertheless, some weeds do not need evolved glyphosate resistance to become a problem in maize and soybean. In biotechnology-related disciplines, “tolerance” is often used as a synonym of “resistance”; Roundup Ready® soybean is described as an herbicide-tolerant plant when actually it has been genetically modified to be resistant to glyphosate (Roy 2004). Tolerance is the *innate* ability of some species to survive herbicide rates that control other weed species in the same

agro-ecosystem (Zelaya and Owen 2005). Weeds may survive herbicides due to reduced herbicide absorption, reduced translocation of the herbicide from the site of absorption to the site of action, metabolic detoxification or altered target site (Koger and Reddy 2005). Weed species can survive glyphosate applications by one or more of these mechanisms; however, other tolerance mechanisms possibly could be revealed. Glyphosate only marginally controls tropical spiderwort in glyphosate-resistant cotton (Culpepper *et al* 2004). Monquero (2004) suggested that one of the tolerance mechanisms to glyphosate in tropical spiderwort could be due to the chemical composition of the epicuticular waxes which contain lipophilic components in higher concentration than other species studied resulting in reduced penetration of the herbicide. Other studies have indicated that climbing dayflower (*Commelina diffusa* L.) had the ability to recover after glyphosate application because of large starch reserves in the leaves (Tuffi-Santos 2004).

Weed population shifts occur due to a differential response of weed species to specific herbicides and other management practices. Under the appropriate selection pressure conditions (e.g., repeated use of a specific herbicide and rate), tolerant weed species may become prevalent in an agro-ecosystem without the process of evolved herbicide resistance. Species such as tall morning-glory (*Ipomoea purpurea*), giant pennyworth (*Hydrocotyle* sp.), tropical spiderwort, Asiatic dayflower and velvetleaf (*Abutilon theophrasti*) have been reported to be more tolerant to glyphosate compared with other weed species (Paulson 2005; Jordan *et al.* 1997; Culpepper *et al.* 2004; Owen and Zelaya 2005).

The introduction of genetically modified crops resistant to glyphosate could result in an accelerated shift to glyphosate-tolerant weeds. The observed tolerance to glyphosate and the extended emergence period makes Asiatic dayflower a weed that is difficult to manage in Roundup Ready<sup>®</sup> soybean and maize (Fawcett 2002). The recurrent use of glyphosate likely releases Asiatic dayflower from competition with other weeds. Inconsistent control of Asiatic dayflower has been reported in a number of Iowa fields planted to GR maize and soybean. Five separate cases in Vinton, Albia and Homestead, IA provide anecdotal evidence that a single application of glyphosate ( $0.84 \text{ kg ae ha}^{-1}$ ) does not provide satisfactory control of Asiatic dayflower and is indicative of Asiatic dayflower glyphosate tolerance. Furthermore, Fawcett (2003) reported that applications of PRE herbicides such as clomazone and flumioxazin, as well as POST applications of bentazon, acifluorfen, lactofen, flumiclorac, imazamox and fomesafen did not provide satisfactory control on Asiatic dayflower.

While the literature describes ecological and botanical research conducted with Asiatic dayflower, there is little research describing the response of Asiatic dayflower to glyphosate and other herbicides. The main objective of this research was to confirm the presumed tolerance of Asiatic dayflower to glyphosate under greenhouse and field conditions. To support the results from greenhouse and field experiments, shikimate accumulation in GR and non-GR maize and soybean after glyphosate treatments was compared to the accumulation in Asiatic dayflower.

## **MATERIALS AND METHODS**

### **Seedling collection and greenhouse conditions**

For the timing and rate response experiment, explained below, Asiatic dayflower seedlings (1 leaf) which were collected from a glyphosate-resistant soybean field in Vinton, IA during spring 2005. The field had been treated in previous years with at least two applications of glyphosate ( $0.84 \text{ kg ae ha}^{-1}$ ). In addition, Asiatic dayflower seeds were collected in 2005 from Vinton IA. Seeds were stored at 5 C for 9 months then stratified at 0 C for 7 days and planted in flats containing greenhouse soil mixture. Plants were tested to measure the response to various glyphosate rates and evaluated for shikimic acid accumulation. A single seedling was placed in 10 cm by 10 cm pot filled with a greenhouse soil mixture containing peat:perlite:loam (1:2:1). Plants were grown in the greenhouse under natural light supplemented with 16 h of  $600 \text{ to } 1000 \mu\text{mol}^{-2} \text{ ms}^{-1}$  PPFD. Plants were maintained at 28/22 C day/night temperature and watered and fertilized as needed.

### **Glyphosate rate response**

The response of Asiatic dayflower to glyphosate (Roundup WeatherMax, Monsanto, St. Louis, MO., USA) was estimated by comparing a de-ionized water ( $\text{dH}_2\text{O}$ ) treatment with glyphosate applied at 0.84, 1.68, 3.36, 6.76 or  $13.44 \text{ kg ha}^{-1}$ . Treatments were applied with a flat-fan nozzle (80015E, Teejet Spraying Systems, Ill., USA) in a  $\text{CO}_2$  powered spray chamber (SB5-66, DeVries Manufacturing, Mn., USA) delivering  $187 \text{ l ha}^{-1}$  at 275 kPa ( $2.8 \text{ kg cm}^{-2}$ ). Plants were treated 21 days after transplanting at the 8 leaf stage. Visual estimates of Asiatic dayflower control



were taken at 21 days after treatment (DAT) based on a scale of 0 (no control based on the  $dH_2O$  treated plants) to 100 (plant death). Shoot biomass was estimated by clipping Asiatic dayflower at the soil level and measuring fresh weight. Shikimic acid accumulation (process described below) was also determined. The experimental design was a randomized complete block with two blocks and each treatment was replicated three times. ANOVA was performed for all the parameters. Means were separated using Fisher's least significant differences test ( $P \leq 0.05$ ). Regression analysis was conducted, and linear and nonlinear models were fit to the data. Logistic equation was used to describe a nonlinear response curve in Asiatic dayflower biomass. Shikimate accumulation data was fitted to the Gompertz equation with 3 parameters. SigmaPlot 8.0. was used in both cases.

### **Timing and rate response experiment**

Herbicide treatments were a factorial arrangement of glyphosate rates over the treatment timings of 2-leaf stage (L), 4 L, 8 L, 16 L and 32 L, with glyphosate rates of 0.21, 0.42, 0.84, 1.68 and 3.36 kg ae ha<sup>-1</sup>. A control check ( $dH_2O$ ) was also included. Visual evaluation of damage after glyphosate application was assessed 21 DAT comparing the damage of the treated plants against the control check, a scale of 0 (no control) to 100 (plant death) was used. The fresh weight of each plant was determined by clipping plants at the soil level 21 DAT. The experimental design was a randomized complete block with two blocks and each treatment was replicated four times. ANOVA was performed for all the parameters. Means were separated using Fisher's least significant differences test ( $P \leq 0.05$ ).

## **Evaluation of herbicides for Asiatic dayflower control under field conditions**

Studies were conducted in grower fields with high populations of Asiatic dayflower. Two fields at Vinton IA in 2005 and, two fields, one at Vinton and one at Humeston IA., in 2006 were included in the experiment. Five PRE and five POST herbicides were applied at the highest rate on the commercial label with the exception of glyphosate which was applied at three rates (Table 1). All POST herbicide applications included label-recommended additives. PRE herbicides were applied during the sowing week and POST herbicides were applied 21 and 42 DAP. Treatments were applied using a CO<sub>2</sub>-pressurized backpack sprayer equipped with flat-fan nozzles calibrated to deliver 185 l ha<sup>-1</sup> at 172 kPa .

Visual evaluations of Asiatic dayflower control after herbicide application were conducted 21 and 42 DAT a scale of 0 (no control, based on untreated plots) to 100 (no Asiatic dayflower establishment or plant death) was used. Additionally, three quadrats 50 cm by 50 cm were placed arbitrarily in each plot to determine weed population density. Plots were 3.0 m wide and 7.6 m in length. The experiments were arranged in a randomized complete block design with three replications and two locations each year. ANOVA was performed for all the parameters. Means were separated using Fisher's least significant differences test ( $P \leq 0.05$ ). Data were analyzed using SAS<sup>®</sup> Proc Mixed. Years and blocks were taken in account as random factors in the model and were pooled (Littell et al. 1996).

### **Shikimate extraction and determination**

Shikimate concentration was measured using the procedure of Cromartie and Polge (2002). Glyphosate was sprayed on the plants through an even flat flow nozzle delivering  $187 \text{ l ha}^{-1}$  at 275 kPa ( $2.8 \text{ kg cm}^{-2}$ ). Glyphosate-treated plants were clipped at soil level, dried at 35C for 48 h in paper bags and stored at 5C for 21 d. The apex area is the most active part of the plant and shikimate is mostly expressed in the growing regions where the tissues are youngest. Dry tip samples (0.2g) of each treated plant were used to estimate endogenous shikimic acid. Samples were initially ground with a mortar and pestle in liquid nitrogen and then ground again in 0.25 N HCl at a ratio of 1:20 (w/v). The extract was centrifuged at 12,000 rpm in a tabletop micro-centrifuge (Marathon 13K/M, Fisher Scientific, PA., USA) for 20 min. and the supernatant was collected and used for the shikimate assay. Ten  $\mu\text{l}$  of the supernatant were mixed with 240 $\mu\text{l}$  of  $\text{D}_2\text{O}$  and 250  $\mu\text{l}$  aqueous solution of 0.5% periodic acid and 0.5% sodium *meta*-periodate (w/w) in a 1.5 ml eppendorf micro-centrifuge tube. The tube was vortexed and heated in a water bath at 37 C for 45 min. After heating, the reaction was quenched with 500  $\mu\text{l}$  of a 3:2 (v/v) 1.0 M NaOH: 0.056 M  $\text{Na}_2\text{SO}_3$ . A 200- $\mu\text{l}$  aliquot of the solution was placed into each well of a 96-well microplate, and the optical density at 380 nm was measured with a microplate reader (Bio-Tek Power Wave XS equipped with KC4 software).

## **Shikimic acid accumulation in Asiatic dayflower, GR maize and soybean and non-GR maize and soybean**

GR and non-GR plants of maize and soybean and Asiatic dayflower were treated with glyphosate in order to compare the relative accumulation of shikimate. The soybean varieties used were GH H-2162 Roundup Ready® and IA 3017 which was non-GR, and DK C58-19 Roundup Ready® and PR 095 non-GR maize hybrids. Shikimate assessment was conducted using the procedure described previously.

Seeds were planted in pots and seedlings were grown under standard greenhouse conditions as previously described and sprayed with four rates of glyphosate ( $\text{dH}_2\text{O}$ , 0.86, 1.72, and 3.44 kg ae ha<sup>-1</sup>) 21 days after emergence. Apex tissue from Asiatic dayflower and soybean, as well as the bottom 3 cm of the shoot in maize were harvested 0 (2 h after application), 7 and 14 DAT and evaluated for shikimate accumulation as previously described. The experimental design was a randomized complete block with two blocks and four replications. ANOVA was performed for all the parameters. Means were separated using Fisher's least significant differences test ( $P \leq 0.05$ ).

## **RESULTS AND DISCUSSION**

### **Asiatic dayflower response to glyphosate in the greenhouse**

Asiatic dayflower control with glyphosate using 0.84 kg ae ha<sup>-1</sup> (1X) was not significantly different from the untreated control. In the absence of glyphosate,

Asiatic dayflower biomass weighed 45 to 60 g fresh weight. These biomass levels decreased sigmoidally when glyphosate rate was increased. Fresh weight decreased to a minimum of 3 to 18 g when Asiatic dayflower was sprayed with a glyphosate rate of 13.76 kg ae ha<sup>-1</sup> (16X) (Figure 3). Only at this rate were dead plants observed (data not shown). However, the differences in Asiatic dayflower biomass reduction among the three highest glyphosate rates (4, 8 and 16X) were not statistically significant ( $\alpha=0.05$ ). Asiatic dayflower control for the 2X glyphosate rate was comparable with 4 and 8X treatments and superior to the common rate used in soybeans 0.84 kg ae ha<sup>-1</sup> (1x) and the untreated control. There was no statistical difference between the 1X glyphosate and the untreated control confirming the tolerance of Asiatic dayflower to glyphosate. Asiatic dayflower treated with 1X glyphosate showed only slight phytotoxicity 14 DAT when compared to the untreated control. This research validates the results obtained by other authors who reported the inconsistent response of Asiatic dayflower to glyphosate (Manabe et al. 1990, Fawcett 2002, Kuhns and Harpster 2005).

### **Asiatic dayflower response to glyphosate does not change regardless of the application rate or the plant growth stage**

Under greenhouse conditions, the control provided by rates of 0.84 kg ae<sup>-1</sup> and less did not control Asiatic dayflower; a rate of 0.84 kg ae<sup>-1</sup> applied at the earliest growth stage (2L) gave just 10% control (Figure 2). High rates (1.72 and 3.44 kg ae ha<sup>-1</sup>) of glyphosate provided better control during the earlier growth stages 2 to 8L. However, the control provided by these rates can not be qualified as satisfactory.

Asiatic dayflower control was only 28% for the highest glyphosate rate applied (3.44 kg ae ha<sup>-1</sup>) at the earliest growth stage (2L). As Asiatic dayflower plants grew, glyphosate control declined. At the latest growth stage evaluated (32L) there were no significant differences among glyphosate rates.

Asiatic dayflower fresh weight was not significantly different among glyphosate rates within growth stages (Figure 2). Plants treated with dosages lower than 0.84 kg ae<sup>-1</sup> were not injured. Plants treated with rates equal and above 0.84 kg ae<sup>-1</sup> demonstrated reductions in fresh weight and also demonstrated some injury symptoms. These symptoms and the decrease in fresh weight were lower in plants treated with glyphosate at the latest growth stages. Park et al. (2004) reported that Asiatic dayflower tolerance to glyphosate was greater when the herbicide was applied at the 3L than the 5L growth stage. In the present study, the results revealed that glyphosate at 0.84 kg ae ha<sup>-1</sup> rate provided 10% control when applied at 2L stage and less than 4% control when applied at the 32L growth stage.

### **Asiatic dayflower response to PRE-herbicides under field conditions**

The standard weed management programs in GR maize-soybean agro-ecosystems have not provided acceptable control of Asiatic dayflower (Fawcett 2002). Similarly, Webster (2006) found that some commonly used PRE herbicides are not useful to control tropical spiderwort emergence in cotton. The most effective control occurred with metribuzin and KIH – 485 (Table 2). Metribuzin provided 80 and 83% Asiatic dayflower control 21 and 42 DAT respectively and also reduced the

number of plants. By 42 DAT, metribuzin still reduced Asiatic dayflower establishment, while Asiatic dayflower continued emerging and growing in the untreated plots. KIH – 485 provided 55% control 21 DAT and control increased to 71% 42 DAT, when compared to the untreated control. The 42 DAT control provided by KIH-485 was comparable to that of metribuzin, and better than the other PRE herbicides evaluated.

Asiatic dayflower control with flumioxazin, flufenacet, and S-metolachlor was lower than metribuzin in all of the cases; however, control provided by these herbicides was similar to KIH – 485 when evaluated 21 DAT (Table 2). Asiatic dayflower control with flumioxazin was equivalent to KIH – 485 evaluated 21 and 42 DAT; this is consistent with the results reported by Fawcett (2002). Webster (2006) stated that flumioxazin control of tropical spiderwort was not adequate; however the rate used in that study ( $0.072 \text{ kg ai ha}^{-1}$ ) was lower than the rate applied in the present experiment ( $0.11 \text{ kg ai ha}^{-1}$ ). Flufenacet and S-metolachlor provided 53% control 42 DAT. These results are similar to flumioxazin but inferior to metribuzin and KIH – 485.

All PRE herbicides reduced Asiatic dayflower emergence compared to the untreated plots. However, there were no important differences in Asiatic dayflower population density among herbicide treatments 21 and 42 DAT (Table 2). Reduced control by some PRE herbicides could have been caused by the long emergence period (May to August) demonstrated by Asiatic dayflower (Takabayashi and Nakayama 1978). Therefore it is important to use PRE herbicides that exhibit long

soil residual activity controlling Asiatic dayflower. Tillage is another factor that may influence Asiatic dayflower. Tillage likely provides for more uniform Asiatic dayflower germination and early homogenous germination could improve PRE herbicides performance controlling Asiatic dayflower.

### **Asiatic dayflower response to POST-herbicides**

POST herbicides were applied at 21 (<8L leaf stage) and 42 (>32L leaf stage) DAP. Most of the POST herbicides achieved better results when applied at 21 DAP with the exception of glyphosate which performed better when applied 42 DAP (Table 3). Cloransulam-methyl applied 21 DAP provided 80 and 65% control 21 and 42 DAT, respectively. Lactofen applied 21 DAP demonstrated 67 and 60% control of Asiatic dayflower 21 and 42 DAT, respectively. Cloransulam-methyl and lactofen applied 21 DAP significantly reduced the Asiatic dayflower populations compared to the untreated control. High rates of glyphosate (1.68 and 2.52 kg ae ha<sup>-1</sup>) also controlled Asiatic dayflower, nevertheless, applications of high rates delayed GR-soybean growth compared with plants in the untreated plots (data not shown).

Glyphosate applied at 0.86 kg ae ha<sup>-1</sup> gave up to 50% control regardless of the application timing. These results are consistent with results reported by Fawcett (2002). Carfentrazone and flumiclorac applied 21 DAP, as well as lactofen and cloransulam-methyl applied 42 DAP gave a little control of Asiatic dayflower and the effect of the application decreased rapidly.



Carfentrazone and flumiclorac applied 42 DAP did not control of Asiatic dayflower. 42 DAT carfentrazone treatments were similar to the untreated control. According to Fawcett (2002), lactofen and flumiclorac did not control Asiatic dayflower; however, in this study, an early application of lactofen controlled this weed up to 67% and reduced weed population significantly.

### **Shikimate accumulates in glyphosate-tolerant Asiatic dayflower**

The procedure of Cromartie and Polge (2002) was utilized to quantify shikimate accumulation in plant tissues. Asiatic dayflower treated with glyphosate demonstrated increased shikimate levels. In the absence of glyphosate, Asiatic dayflower contained shikimate concentrations of 0.4 to 0.8 mg g<sup>-1</sup> of dry tissue. These levels increased sigmoidally with increasing glyphosate rates to a maximum of 1.8 to 2.2 mg g<sup>-1</sup> of dry tissue when Asiatic dayflower was sprayed with a glyphosate rate of 13.76 kg ae ha<sup>-1</sup> (Figure 3). There were no significant differences ( $\alpha=0.05$ ) in shikimate levels among the untreated control and plants treated with 1, 2 and 4X glyphosate rates. Only plants treated with 8 and 16X glyphosate rates demonstrated shikimate accumulation that was statistically greater than the untreated control. Shikimate accumulation is an indicator of glyphosate inhibition of the shikimic acid pathway (Gout et al. 1992, Haring et al. 1998). Based on previous studies with GR and non-GR crops, the accumulation of shikimate in a glyphosate-tolerant plant was unexpected (Henry et al. 2005, Pline et al 2002). These results confirm that glyphosate caused EPSPS inhibition in Asiatic dayflower; however, this inhibition was not sufficient to cause plant death. Furthermore, shikimate

accumulation in Asiatic dayflower suggested that the tolerance mechanism of this weed to glyphosate was not likely reduced glyphosate absorption, translocation or metabolic detoxification.

To confirm the unexpected shikimate accumulation in Asiatic dayflower, the same procedure was used to compare shikimate levels in Asiatic dayflower, GR maize and soybean, and non-GR maize and soybean (Figure 4). As expected, shikimate accumulation was easily detected in non-GR maize and soybean, regardless of the glyphosate rate applied or the DAT that samples were evaluated. Shikimate accumulation was a little greater at 7 than 14 DAT in non-GR soybean and Asiatic dayflower. The increase in shikimate levels 0 DAT was proportional to the glyphosate rate applied to Asiatic dayflower plants. Although non-GR maize and soybean and Asiatic dayflower demonstrated shikimate accumulation, only Asiatic dayflower survived the application of glyphosate at 1.68 and 3.36 kg ha<sup>-1</sup> and demonstrated just slight symptoms when treated with 0.84 kg ha<sup>-1</sup>. Asiatic dayflower tolerance to glyphosate proved to be significantly greater than for non-GR maize, corroborating the results obtained by Park et al. (2004). Shikimate accumulation was not detected in GR maize and soybean. The accumulation of shikimate suggests that the tolerance mechanism of Asiatic dayflower to glyphosate is not due to a glyphosate insensitive EPSPS. If an insensitive EPSPS was present, shikimate accumulation in the plant would not be observed, based on the results obtained with GR maize and soybean. This is not the first report of shikimate accumulation in plants that survived glyphosate. Mueller et al. (2003) reported that shikimate

accumulates similarly in both GR and non-GR horseweed in response to glyphosate 2 DAT. While the mechanism of glyphosate-tolerance in Asiatic dayflower was not specifically identified, the data supports several possible theories including multiple EPSPS genes encoding various EPSPS isozymes, the existence of glyphosate oxidase reductase (GOX)-like enzyme or the presence of an altered EPSPS (Mueller et al. 2003). However, with multiple forms of EPSPS or with an altered form of EPSPS, shikimate accumulation would be improbable. The existence of GOX enzymes in wild plants has not been reported, as the GOX genes were isolated from bacteria and then introduced into some plants to enhance glyphosate resistance (Mannlerof et al. 1997). Shikimate accumulation in Asiatic dayflower, even after 14 DAT, demonstrates that glyphosate was still active and has not been degraded. Nevertheless, these hypotheses can not be discarded.

Other hypotheses could be drawn from these results. As Asiatic dayflower plants mature, the inherent amount of shikimate in cells increases without any glyphosate application. Thus Asiatic dayflower EPSPS, even though it is sensitive to glyphosate, may not be as efficient as in other plants. In addition the possibility of altered translocation can not be discarded.

## **CONCLUSIONS**

Greenhouse and field experiments provided conclusive evidence of tolerance of Asiatic dayflower to glyphosate regardless of glyphosate rate or the phenological stage of the plant at application. Under greenhouse conditions, single applications of

glyphosate ( $0.84 \text{ kg ae ha}^{-1}$ ) did not affect growth in Asiatic dayflower. High rates of glyphosate reduced plant development. However, only extremely high rates of glyphosate ( $13.44 \text{ kg ae ha}^{-1}$ ) caused death of Asiatic dayflower. High rates of glyphosate ( $3.36 \text{ kg ae ha}^{-1}$ ) applied to Asiatic dayflower at the 2 leaf stage provided just 28% control and reduced plant biomass by less than 50% when compared to the untreated control.

Under field conditions, glyphosate ( $0.84 \text{ kg ae ha}^{-1}$ ) did not control Asiatic dayflower. Metribuzin gave up to 80% control during the two years of experiments. KIH – 485 also provided good control of this weed. Based on the results of this research, the application of PRE herbicides with long residual activity and efficacy on Asiatic dayflower are crucial for the management of this problematic weed. Early applications (21 DAP) of cloransulam-methyl and lactofen provided the best control among the POST herbicides. For many of the POST herbicides, there were no important differences between application timings.

Glyphosate increased shikimate levels in non-GR plants and maize and soybean plants died 14 to 21 DAT. Although glyphosate-treated Asiatic dayflower demonstrated shikimate accumulation, none of the plants died at the glyphosate rates evaluated. This shikimate accumulation was unexpected and the tolerance mechanism of Asiatic dayflower to glyphosate was not identified.

Asiatic dayflower will continue to be a troublesome weed in Iowa GR maize and soybean fields. This study demonstrated that Asiatic dayflower can survive and

thrive in glyphosate-resistant maize and soybean. This research reported shikimate accumulation in Asiatic dayflower. However, the mechanism of glyphosate tolerance in this plant was not determined. Additional studies are necessary to determine the mechanism for glyphosate tolerance in Asiatic dayflower.

In this study Asiatic dayflower plants received just one application of herbicide, but many growers apply herbicides more than once during the growing season. Therefore, it will be necessary to conduct other experiments to determine the response of Asiatic dayflower to multiple herbicide applications. Moreover, combined applications of PRE and POST herbicides should be evaluated. To find other optimum management systems for Asiatic dayflower, it is important to determine how agronomical factors such as tillage, crop density and planting dates influence Asiatic dayflower emergence and growth.

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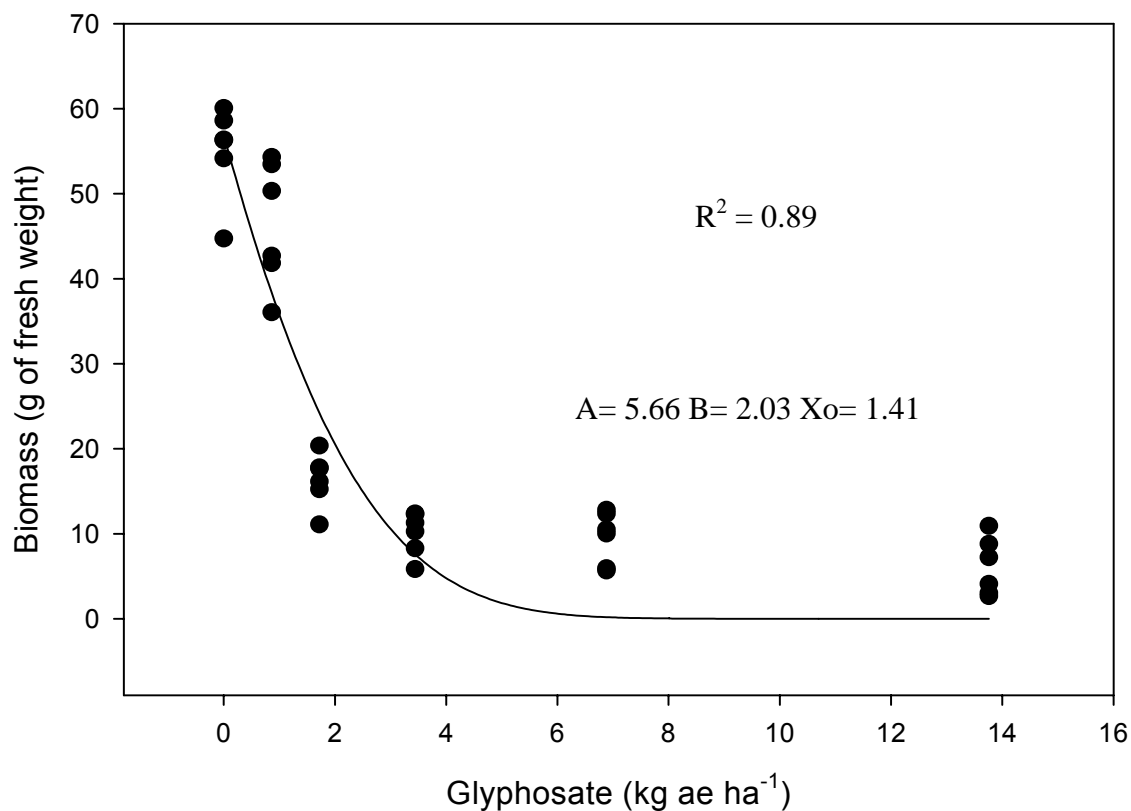
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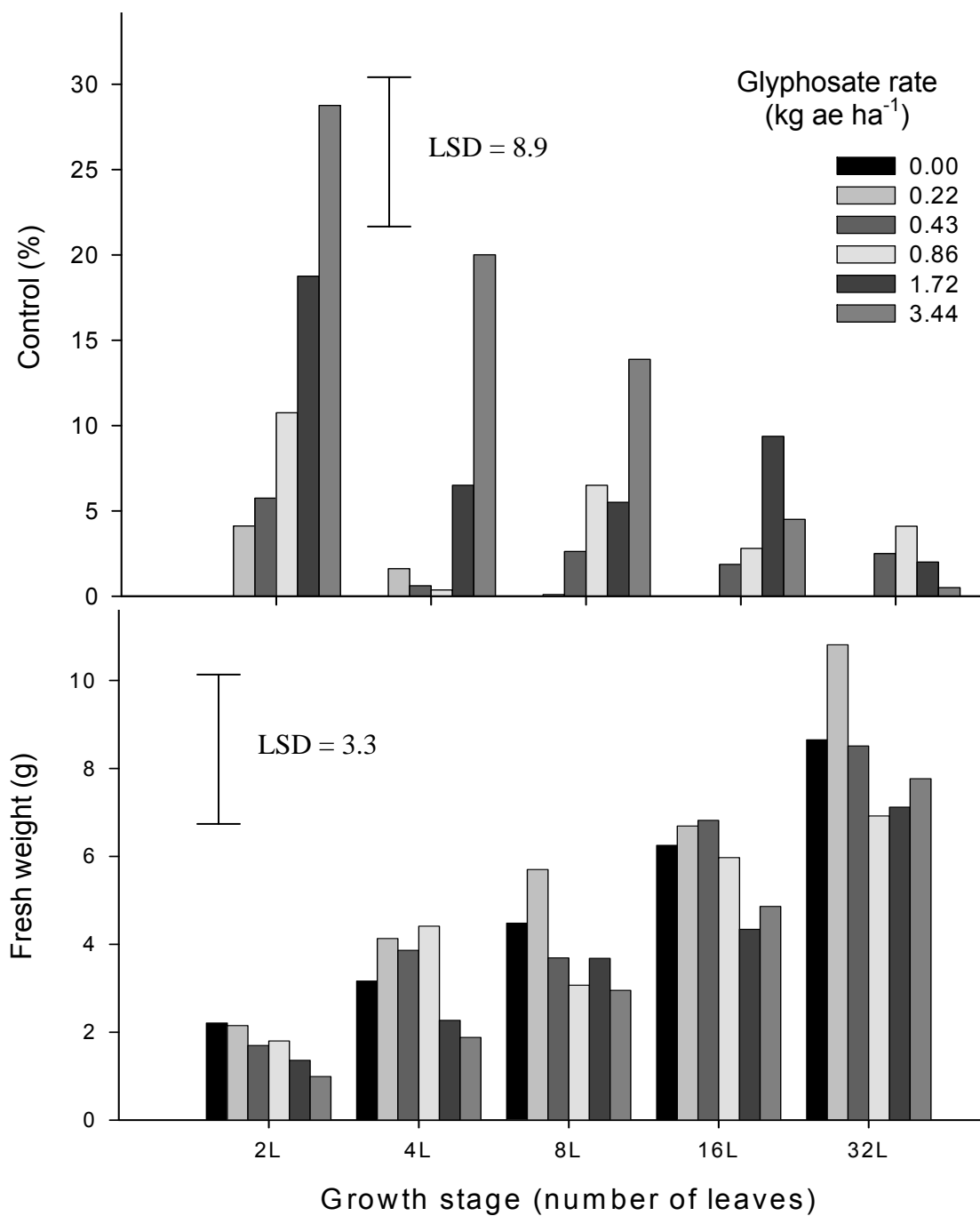
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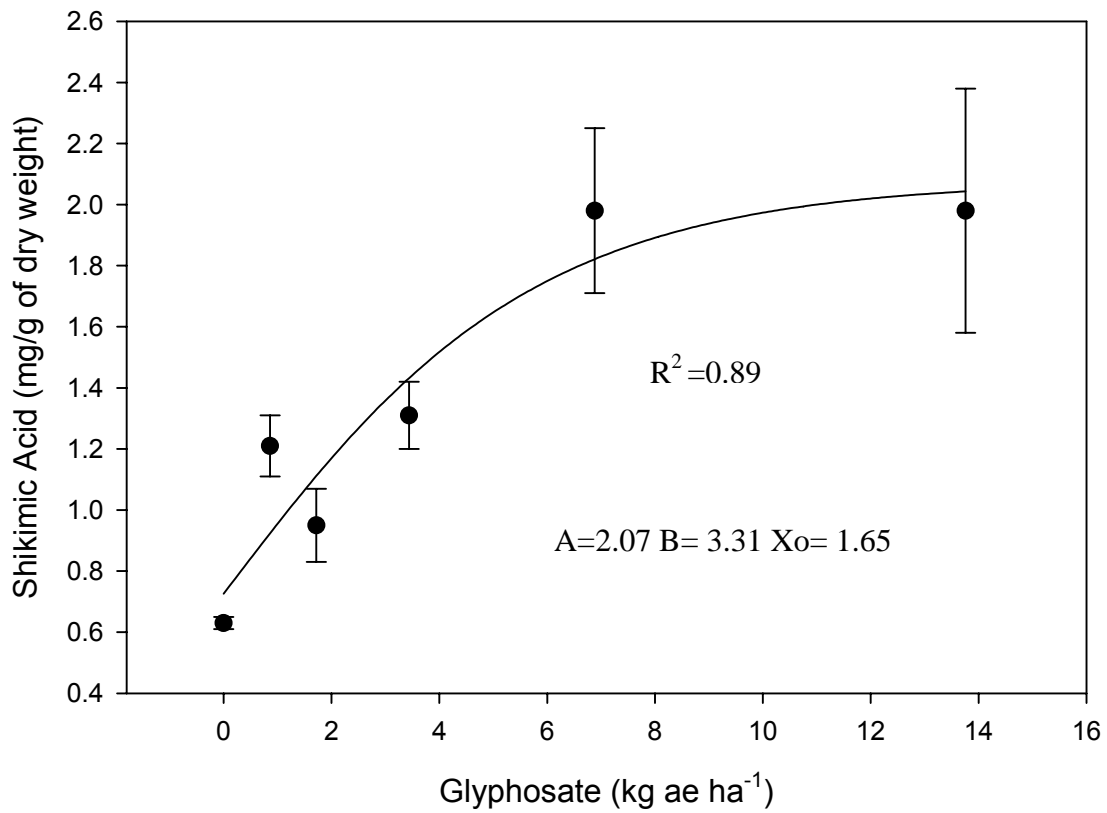
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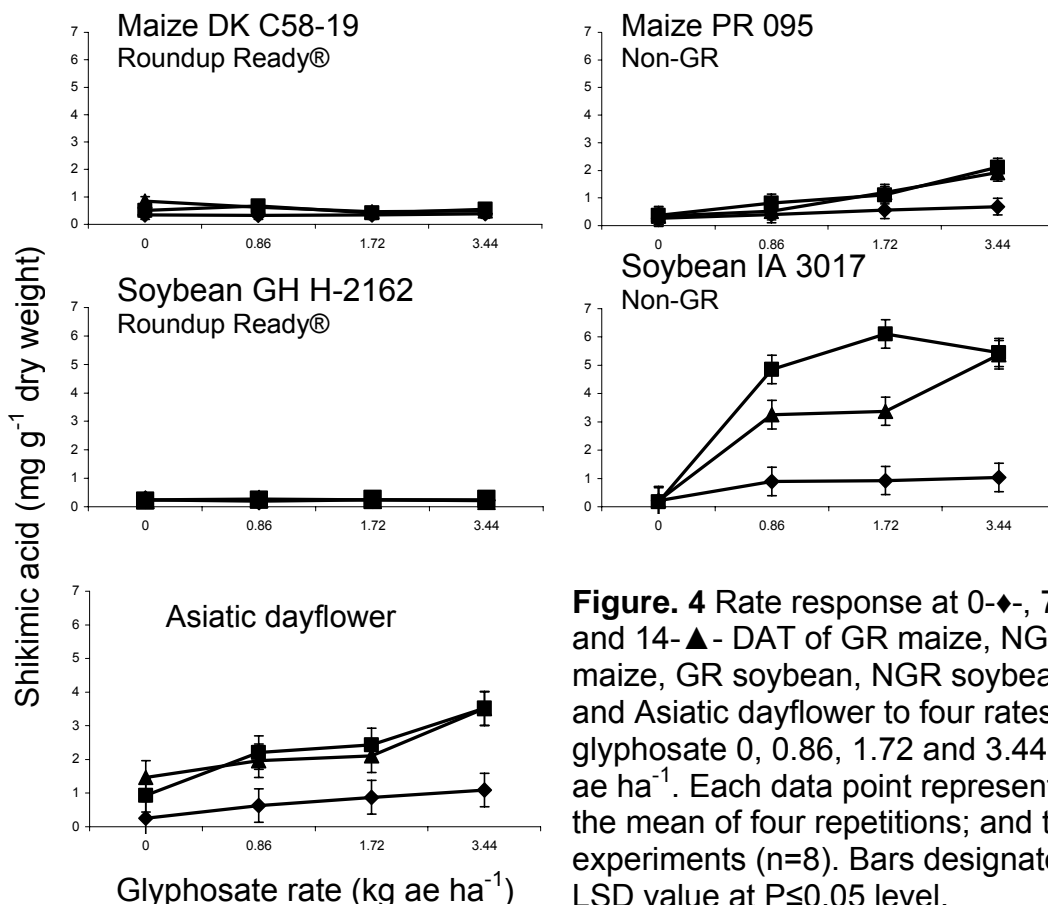
**Figure 1.** Asiatic dayflower biomass in response to glyphosate treatments under greenhouse conditions 21 days after treatment (DAT). Glyphosate was applied at 8L leaf-stage. Each treatment was replicated three times and the experiment was repeated (n=6). Data were fitted to the logistic equation with 3 parameters.



**Figure 2.** Effect of glyphosate rate and growth stage on Asiatic dayflower control and fresh weight under greenhouse conditions. Each column represents the mean of four replications and two experiments ( $n=8$ ). Bars designate LSD values at  $P \leq 0.05$  level.



**Figure 3.** Shikimic acid accumulation in Asiatic dayflower in response to glyphosate treatments under greenhouse conditions 21 days after treatment (DAT). Glyphosate was applied at 8L leaf-stage. Each data point represents the mean of three replications and two experiments ( $n=6$ ) conducted at different times. Data were fitted to the Gompertz equation with 3 parameters. Extensions on symbols designate the standard error associated with individual means ( $S_E$ ).



**Figure. 4** Rate response at 0-♦-, 7-■- and 14-▲- DAT of GR maize, NGR maize, GR soybean, NGR soybean and Asiatic dayflower to four rates of glyphosate 0, 0.86, 1.72 and 3.44 kg ae ha<sup>-1</sup>. Each data point represents the mean of four repetitions; and two experiments (n=8). Bars designate LSD value at P≤0.05 level.

**Table 1.** Herbicides and additives used in field experiments.

Herbicide	Type	Rate (kg ai ha <sup>-1</sup> )	Additives
Metribuzin	PRE	0.84	No additives
S-Metolachlor	PRE	2.14	No additives
KIH - 485	PRE	0.21	No additives
Flufenacet	PRE	0.49	No additives
Flumioxazin	PRE	0.108	No additives
Carfentrazone	POST <sup>1</sup>	0.0066	NIS 0.25% (v/v)
Lactofen	POST	0.22	COC 0.50% (v/v)
Fumiclorac	POST	0.1	COC 2.34 l ha <sup>-1</sup>
Cloransulam	POST	0.0176	NIS 0.25% (v/v)
Glyphosate	POST	0.86	AMS 5% (v/v)
Glyphosate	POST	1.74	AMS 5% (v/v)
Glyphosate	POST	2.6	AMS 5% (v/v)

<sup>1</sup>POST herbicides were applied 21 and 42 DAP.



**Table 2.** Effect of PRE herbicides on Asiatic dayflower control and plant population density 21 and 42 days after treatment (DAT) at Humeston and Vinton IA, 2005 and 2006.

Herbicide	Rate (kg ai ha <sup>-1</sup> ) <sup>2</sup>	Control (%) <sup>1</sup>		Plants (m <sup>2</sup> )	
		21 DAT	42 DAT	21 DAT	42 DAT
Metribuzin	0.84	80 a <sup>3</sup>	83 a	13 a	30 a
S-Metolachlor	2.14	46 b	53 c	32 b	33 ab
KIH - 485	0.21	55 b	71 ab	25 ab	27 a
Flufenacet	0.49	52 b	53 c	29 ab	37 ab
Flumioxazin	0.11	42 b	58 bc	31 ab	50 b
Control		No control	No control	51 c	76 c

<sup>1</sup>Each number represents the mean of three replications and four fields (n=12).

<sup>2</sup>Most common rate used in soybean crops.

<sup>3</sup>Means within a column followed by the same letter are not significantly different.

**Table 3.** Effect of POST herbicides on Asiatic dayflower control and plant population density 21 and 42 days after treatment (DAT) at Humeston and Vinton IA, 2005 and 2006.

Herbicide	Rate (kg ai ha <sup>-1</sup> )	Application timing DAP <sup>1</sup>	Control (%) <sup>2</sup>		Plants (m <sup>2</sup> )	
			21 DAT	42 DAT	21 DAT	42 DAT
Carfentrazone	0.01	21	43 de <sup>3</sup>	25 hi	66 ef	71 de
Lactofen	0.22	21	67 bc	60 bc	28 a	41 a
Fumiclorac	0.10	21	43 de	35 gh	43 ab	62 cd
Cloransulam	0.02	21	80 a	65 ab	29 a	45 ab
Glyphosate	0.86	21	62 cd	45 fg	49 cd	62 cd
Glyphosate	1.74	21	62 cd	47 ef	48 bc	59 cd
Glyphosate	2.58	21	67 bc	50 de	36 ab	65 cd
Carfentrazone	0.01	42	33 f	13 i	69 ef	91 e
Lactofen	0.22	42	41 de	28 h	73 ef	75 de
Fumiclorac	0.10	42	35 f	30 h	80 f	79 de
Cloransulam	0.02	42	52 de	48 ef	62 ef	59 cd
Glyphosate	0.86	42	58 cd	52 cd	57 de	73 de
Glyphosate	1.74	42	63 cd	66 ab	60 ef	60 cd
Glyphosate	2.58	42	75 ab	72 a	35 ab	50 bc
Control			No control	No control	76 f	83 e

<sup>1</sup> Post herbicides were applied 21 and 42 days after planting (DAP).

<sup>2</sup> Each number represents the mean of three replications and four fields (n=12).

<sup>3</sup> Means within a column followed by the same letter are not significantly different.

*CHAPTER 3: GENERAL CONCLUSIONS AND RECOMMENDATIONS***Conclusions**

- Greenhouse and field experiments provided conclusive evidence of glyphosate tolerance in Asiatic dayflower regardless of the phenological stage of the plant or glyphosate rate.
- Under greenhouse conditions, a single application of glyphosate 0.84 kg ae ha<sup>-1</sup> did not affect growth or shikimate accumulation in Asiatic dayflower.
- Glyphosate applied at 13.44 kg ae ha<sup>-1</sup> killed 50% of the Asiatic dayflower population in the greenhouse.
- Under field conditions, glyphosate does not control Asiatic dayflower.
- Metribuzin PRE gave up to 80% control during two years of experiments.
- Control provided by KIH-485 was statistically similar to metribuzin.
- An early application (21 DAP) of cloransulam-methyl provided 80% control 21 DAT of Asiatic dayflower.
- Lactofen applied 21 DAP control was statistically similar to an early application of cloransulam-methyl.
- Glyphosate increases shikimate levels in non-GR maize and soybean, and in Asiatic dayflower.
- Shikimate did not accumulate in GR maize and soybean.
- Asiatic dayflower will continue to be a troublesome weed in Iowa. This study demonstrated that Asiatic dayflower will survive and thrive in glyphosate-resistant maize and soybean.

### **Recommendations for future research**

In this study, Asiatic dayflower plants received just one application of herbicide, but there are growers that apply herbicides more than once during the growing season. Therefore, it will be necessary to conduct other experiments to determine the response of this weed after two or more herbicide applications. Moreover, combined applications of PRE and POST herbicides should be evaluated. In addition, mixtures of two or more herbicides to control Asiatic dayflower should be assessed. Finally, it will be important to determine how factors such as tillage, crop density and planting dates could be managed to control the emergence and growth of Asiatic dayflower.