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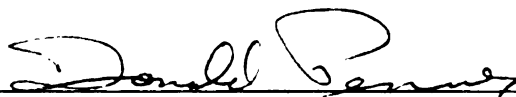
INTERACTION EVALUATION OF GLYPHOSATE,  
GLUFOSINATE, CHLORIMURON AND  
THIFENSULFURON COMBINATIONS

presented by

Rachel Kaye Bethke

has been accepted towards fulfillment  
of the requirements for the

M.S. degree in CROP AND SOIL SCIENCE

  
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**INTERACTION EVALUATION OF GLYPHOSATE, GLUFOSINATE,  
CHLORIMURON AND THIFENSULFURON COMBINATIONS**

**By**

**Rachel Kaye Bethke**

**A THESIS**

**Submitted to  
Michigan State University  
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for the degree of**

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## **ABSTRACT**

### **INTERACTION EVALUATION OF GLYPHOSATE, GLUFOSINATE, CHLORIMURON AND THIFENSULFURON COMBINATIONS**

By

Rachel Kaye Bethke

The stacking of genes to provide resistance to several herbicides previously injurious to a crop provides new opportunities for control of a larger range of species and herbicide resistant weeds. Studies were conducted in the field in 2008 and 2009 and in the greenhouse from 2008 to 2010 to evaluate the combination of glyphosate and glufosinate and the sulfonylurea herbicides, chlorimuron and thifensulfuron on four weeds prevalent in Michigan cropping systems; common lambsquarters, velvetleaf, giant foxtail and Canada thistle. Antagonism was observed with combinations of glyphosate and glufosinate, glyphosate and chlorimuron and glufosinate and both sulfonylureas. Fluorescence measurements of leaves of the treated plants were taken in the greenhouse at 2, 4, 6, 8, 24, 48 and 72 hours after treatment to determine the time of herbicides injury to the plant photosynthetic system. Fluorescence parameters showed glufosinate acted within 2 hours after treatment and glyphosate within 24 hours after treatment. Absorption and translocation of  $^{14}\text{C}$ -glyphosate and  $^{14}\text{C}$ -chlorimuron was examined in the greenhouse to determine the basis for the observed interactions. The addition of chlorimuron increased absorption and translocation of  $^{14}\text{C}$ -glyphosate and the addition of glyphosate to  $^{14}\text{C}$ -chlorimuron also increased absorption and either decreased or had no effect on translocation out of the treated leaf.

**Dedication Page**

This thesis is dedicated to my father, John Carl Bethke. Whom instilled in me a love of plants and agriculture. I am ever grateful to be your daughter.

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## **CHAPTER 1**

### **LITERATURE REVIEW**

Genetically modified crops resistant to glyphosate, glufosinate, and the sulfonylurea herbicides, will soon be commercially available. Hybrids already on the market include glyphosate and glufosinate stacked resistance under the trade name Herculex™ by Dow Agrosciences (Anonymous 2010a). Dow Agrosciences has also released SmartStax™ corn hybrids with Monsanto for the 2010 growing season. It is the first eight-gene stacked corn hybrid, with herbicide resistance to both glyphosate and glufosinate (Anonymous 2008). Du-Pont Pioneer is currently developing Optimum®GAT® (356043) soybean which will be resistant to glyphosate and the sulfonylurea herbicides. This new trait has been under field test since 2003 and is set to release in 2013 or 2014 (Willits 2009). The GAT® gene (glyphosate acetyltransferase) catalyzes the acetylation of glyphosate to the inactive N-acetyl glyphosate (NAG) form (Siehl et al. 2007). Pioneer is also developing a corn hybrid (98140) with Optimum®GAT® technology including resistance to glufosinate still pending approval (Willits 2009). Resistance to these herbicides will give producers new opportunities for herbicide management systems. However, the tank-mixing of these herbicides could result in unexpected interactions such as reduced, improved, or similar weed control compared to the herbicides applied alone.

## THE IMPACT OF HERBICIDE RESISTANT WEEDS

Plants can generally react in one of three ways to the application of an herbicide; susceptibility, tolerance or resistance (LeBaron and Gressel 1982). Susceptibility occurs when a plant is treated with a herbicide and becomes injured. Generally, tolerance and resistance refer to conditions where the plants withstand herbicide applications (Gressel 1985). Tolerance is referred to as the natural occurring non-phytotoxic response of a weed species or crop to a specific herbicide (Holt and Lebaron 1990). Resistance often involves the concept of selection of a physiological mechanism in a weed after repeated exposure to an herbicide. Herbicide resistance was first reported by Switzer for 2,4-D in wild carrot (Switzer 1952).

To date there are 347 herbicide resistant biotypes in 195 species occurring in over 340,000 fields across the world (Heap 2009a). Prior to development of herbicide resistance, many of these weed species were difficult to control, and present an even greater challenge for economically sound management. Worldwide, 108 weed species have been confirmed resistant to ALS-inhibiting herbicides, and eighteen species have been confirmed resistant to glyphosate (Heap 2009a). The newest weed in the United States with confirmed resistance to glyphosate is giant ragweed (*Ambrosia trifida* L.), found in a population in Tennessee (Norsworthy et al. 2010).

The normal variability in response of weeds to a herbicide can allow populations of weeds with enhanced tolerance to increase with increased use of

the herbicide even though the weed can still be controlled at a higher rate (Owen and Zelaya 2005). Shifts in weed populations; either to resistant populations, or other tolerant species, due to overuse of one mode of action (MOA) have been observed in a few studies (Davis et al. 2009; Heard et al. 2003; Hugh and Reboud 2009; Wilson et al. 2009). Repeated use of glyphosate over 6 years led to a population shift from populations of kochia (*Kochia scoparia* L. Schrad.) and wild proso millet (*Panicum miliaceum* L. ) to populations of common lambsquarters (*Chenopodium album* L. ) (Wilson et al. 2009). After four years of non-glyphosate postemergence herbicide use, the ratio of glyphosate resistant horseweed (*Conyza Canadensis* L. Cronq.) to glyphosate susceptible biotypes shifted from 3:1 to a ratio of 1:6 (Davis et al. 2009). A four year experiment in Canada showed that applying ALS inhibiting herbicides increased the occurrence of the resistant weed biotypes (Hugh and Reboud 2009).

Resistance to multiple herbicides is also becoming a large problem in several areas. Common waterhemp (*Amaranthus rudis* Sauer) resistant to both glyphosate and thifensulfuron has been confirmed in Missouri (Legleiter and Bradley 2008). Common waterhemp also has been confirmed resistant to the protoporphyrinogen oxidase (PPO) - inhibiting herbicides such as lactofen, fomesafen and acifluorfen (Legleiter and Bradley 2008; Patzoldt et al. 2005). To control weeds that are resistant to multiple herbicides it is important to use different herbicide chemistries to achieve complete control so the resistant genes will not persist in the seedbank.

## WEEDS OF INTEREST

Weeds are a problem in all cropping situations. Producers work diligently to protect their investment by using methods of weed control such as applying herbicides to control competitive weeds that can be detrimental to crop yields. Knowledge of herbicide programs specific to a farmers weed problem is necessary to ensure adequate control and protect the environment. Michigan producers are generally confronted with weeds adapted to the Michigan environment. Information on weed density thresholds for specific species and optimum height for control is valuable when developing an herbicide program. The weeds studied for this research were chosen because they are common weeds in Michigan agronomic systems. Two annual broadleaves; common lambsquarters (*Chenopodium album* L.) and velvetleaf (*Abutilon theophrasti* Medik.); an annual grass species; giant foxtail (*Setaria faberi* Herm.), and the perennial; Canada thistle (*Cirsium arvense* L. Scop.), were chosen to be the focus of this study.

**Common Lambsquarters (*Chenopodium album* L.)** Common lambsquarters is a summer annual broadleaf weed which belongs to the goosefoot (*Chenopodiaceae*) family. It is a worldwide problem and considered one of the most important weeds in agriculture (Heap 2009a). It is native to Europe and Asia, and is one of the five most widely distributed weeds (Holm et al. 1977). Common lambsquarters is a problem due to the species high fecundity; producing over 70,000 seeds per plant which can



persist up to 78 years in the soil. The dimorphic characteristics of common lambsquarters seed results in a relatively germinable thin-walled brown seed that will germinate the next year and a darker seed that exhibits primary dormancy (Harper et al. 1970). Common lambsquarters also may hybridize with the other *Chenopodium* species, opening the door for new genetic populations and subsequently a variety of subspecies, varieties and forms (Abrams 1944).

Common lambsquarters has populations that are resistant to a variety of herbicide Modes of Action across the country and the world. The first report of triazine (photosystem II (PSII) inhibitor) resistance was common lambsquarters in 1975 in Michigan; since that time 18 countries have reported PSII inhibitor resistance (Heap 2009a). Common lambsquarters has also exhibited resistance to the ALS-inhibitors; thifensulfuron and imazamox in Michigan, Ohio, and Ontario (Heap 2009a).

Resistance to glyphosate has not been reported for common lambsquarters. However, producers and extension agents have reported problems with controlling common lambsquarters with glyphosate in the Midwest (Owen and Zelaya 2005; Wilson et al. 2009). These studies attributed the shift of weed population to glyphosate tolerant biotypes of common lambsquarters to the natural variability in tolerance and repeated use of low rates of glyphosate. However, this study also found that weed populations shifted to common lambsquarters even when no glyphosate was applied, telling that common lambsquarters is a very competitive weed even without herbicide resistance.

**Velvetleaf (*Abutilon theophrasti* Medik.)** Velvetleaf is a summer annual which belongs to the Malva (*Malvaceae*) family. Velvetleaf is a very competitive weed and is especially a problem in soybean production (Eaton et al. 1976; Hagood et al. 1980). Velvetleaf was introduced from China in the 1700's as a fiber crop (Spencer 1984). However, due to the lack of good fiber quality velvetleaf was not readily used. It became a successful weed due to the lack of control, seed dormancy, high germination depths, and tolerance to herbicides (Spencer 1984). Until glyphosate was introduced, velvetleaf was one of the most common weed problems in corn, soybean, and cotton in the United States due to the low efficacy of the preplant and preemergence herbicide options (Harvey et al. 1977; Herr and Stroube 1970).

Velvetleaf has been confirmed resistant to the triazines, which was first reported in 1984 in Maryland and reported in 2004 in Michigan (Heap 2009a). The mechanism for resistance is increased herbicide metabolism due to enhanced glutathione s-transferase activity (Anderson and Gronwald 1991). Velvetleaf has also been reported to have differential tolerance to glyphosate but no known resistance has been reported (Kapusta et al. 1994).

**Giant Foxtail (*Setaria faberi* Herrm.)** Giant foxtail is a summer annual grass in the grass (*Poaceae*) family which reproduces by seed. It is a native of

eastern Asia (Wang et al. 1995a; Wang et al. 1995 b) and has expanded due to human influence (Haflinger and Scholz 1980). It is an economically important weed and is considered a nearly monomorphic species (Wang et al. 1995a; Wang et al. 1995 b) having only slight biotype differentiations in adaxial leaf blade pubescence (Pohl 1962). It is a C<sub>4</sub> plant and competes efficiently because of its plastic growth (Dekker 2003). Giant foxtail is extremely competitive in corn reducing yields 13-14% with only three plants per foot of a row in Michigan (Anonymous 2005). Allelopathic effects of giant foxtail have been found to reduce corn growth (Bell and Koeppe 1972).

Resistance to the triazine herbicides in giant foxtail was first confirmed in 1984 in Maryland and most currently with resistance to the ALS inhibitors in 2006 in Michigan (Heap 2009a). Giant foxtail has also been found with resistance to the ACCase inhibitors in Wisconsin and Iowa (Heap 2009a). There is no confirmed glyphosate resistance in giant foxtail.

**Canada thistle (*Cirsium arvense* L. Scop.)** Canada thistle, a member of the Aster (*Asteraceae*) family, is a perennial weed that grows from seed and underground roots. It is considered a potential model weed due to the weedy characteristics, small genome, cDNA library, adaptability to a wide range of conditions, distribution, and economic impact (Chao et al. 2005). This species has significant ecotype differences depending on location of root stock source with differences in leaf, seed and flower morphology,

which can lead to differential response of herbicides (Hodgson 1964).

Proper control and management of Canada thistle requires that the roots of the plant be killed or somehow removed. Shoot elongation and flowering are induced with a 16h photoperiod. Without this photoperiod they remain low growing rosettes (Hunter and Smith 1972). The physiological stage of growth has been important for translocation of systemic herbicides to the roots; more glyphosate is translocated to the roots when applied at the rosette stage than the bud stage (Hunter 1995).

The only reports of herbicide resistance in Canada thistle were to the synthetic auxins in 1979 and 1985 in Sweden and Hungary, respectively (Heap 2009a).

## **HERBICIDES OF INTEREST**

**Glyphosate.** Glyphosate (N-(phosphonomethyl)-glycine) is a broad-spectrum organophosphorus postemergence herbicide that inhibits the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSP). This enzyme is involved in the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan as well as secondary plant products (Amrhein et al. 1980; Jaworski 1972). Inhibition of EPSP synthase is competitive with respect to phosphoenolpyruvate and uncompetitive with respect to shikimate-3-phosphate (Boocock and Coggins 1983). This inhibition leads to an accumulation of shikimate in the primary vacuole of glyphosate-

treated tissue (Hollander-Czytko and Amrhein 1983). Observed glyphosate phytotoxicity in plants is not only due to the inhibition of aromatic amino acids (Duke 1985), but also inhibits the accumulation of a chlorophyll precursor; 5-aminolevulinic acid (ALA), which results in reduced chlorophyll synthesis (Kitchen et al. 1981) and a decrease in all porphyrin enzymes (Hoagland and Duke 1982). The cause of this reduced ALA accumulation is thought to be due to a reduction of  $\alpha$ -ketoglutarate production by glyphosate (Hoagland and Duke 1982). Glyphosate has a general effect on all plant cellular structures with which it comes in contact. These general effects include; swelling of the chloroplast envelope and thylakoids, and swelling of the endoplasmic reticulum (Pihankaski and Pihankaski 1980). Glyphosate also has a feed-back inhibition on phenylalanine ammonia-lyase (PAL), an enzyme in the synthesis of the amino acids phenylalanine and tyrosine. Glyphosate decreases their synthesis, which leads to decreased synthesis of phenolic compounds resulting in decreased feed-back inhibition of PAL synthesis (Duke and Hoagland 1984).

Glyphosate sensitive weeds exhibit early symptomology of chlorosis followed by necrosis usually from 2 to 10 days after application (Baird et al. 1971). Injury symptoms generally appear first on immature leaves and at growing points (Baird et al. 1971). Glyphosate gives a wide range of control of annual and perennial weeds and generally is considered very phytotoxic to annual grasses (Sprinkle

1974). After what seems like complete necrosis and leaf drop, compensatory growth of the weeds can occur. This positive response of plants to injury is found commonly in perennial and woody species but also is exhibited as witch's broom in grasses and meristematic regrowth in dicots. This can result in greater than expected dry weights than controls (Belsky 1986). Glyphosate resistant crops allow selected glyphosate formulations to be used postemergence throughout the growing season.

*Glyphosate efficacy on weeds of interest.* Glyphosate is a non-selective herbicide; however certain weed species are inherently tolerant to glyphosate. Velvetleaf exhibits a low level of tolerance to glyphosate (Kapusta et al. 1994), whereas the Poacea species like giant foxtail, are susceptible (Sprankle 1974). Velvetleaf's tolerance to glyphosate has been attributed to an elevated EPSP synthase level in many species that will compensate for the glyphosate induced reduction of EPSP synthase (Amrhein et al. 1983). Common lambsquarters has been shown to have differential susceptibility to glyphosate application, the reason is not entirely known. Some have reported differences in control based on growth stage (Jaworski 1972; Lich et al. 1997; Ziska et al. 1999) and others have reported no differences between growth stages in response (Sikkema et al. 2004; Tharp et al. 1999). These differences have been attributed to different biotypes at these locations (Schuster et al. 2007). Glyphosate efficacy on Canada thistle is dependent upon the stage of growth, with more injury occurring when controlled in the rosette stage than at bud initiation (Hunter 1995, 1996).

**Glufosinate.** Glufosinate (2-Amino-4-(hydroxy-methyl-phosphoryl)butanoic acid) is a broad-spectrum, postemergence herbicide that results in necrosis of the plant tissue on contact. Glufosinate is a glutamine synthetase (GS) enzyme inhibitor in sensitive species inhibiting the production of glutamine (Hoagland 1983; Lea et al. 1984). Glutamine synthetase converts glutamate and ammonia to glutamine. Inhibition causes accumulation of toxic ammonia in the cells (Manderscheid and Wild 1986; Wild et al. 1987). However this ammonia build up is not what is directly responsible for glufosinate toxicity, the ammonia has been thought to directly affect photosynthesis (Ridley 1989) but when ammonia alone is added, photosynthesis is not inhibited (Wild et al. 1987). Alternative evidence shows that photosynthesis is inhibited by an accumulation of glyoxylate inhibiting ribulose-1,5 bis-phosphate carboxylase/oxygenase (rubisco) (Wendler 1992; Wild 1993). Glufosinate has no herbicidal activity unless applied directly to the foliage, increasing the spray carrier volume helps increase coverage (Smith 1988a, 1988b). The FINALE formulation of glufosinate was introduced by Bayer Crop Sciences in 1994 and discovered in 1981 (Sensemen 2007). The herbicide was primarily used as a burn-down herbicide before crop planting in reduced tillage systems and for weeds in right-of-ways and other non-crop areas (Smith 1988b).

Sensitive species exhibit chlorosis and wilting occurs within 3-5 days after application with necrosis developing after 1-2 weeks. The symptomology is similar to that of the PSII inhibitors and will not occur unless light is present (Hess 2000; Hoagland 1983). Rate of symptomology after application increases with bright sunlight, high humidity, and moist soil conditions. Under these conditions

injury can be seen by 2 days after treatment (Al-Khatib et al. 2003) The contact herbicide has little movement in the xylem and phloem although greater translocation is found in the grass species than broadleaf herbicides (Steckel et al. 1997a). Translocation of glufosinate can be increased if applied at high relative humidity (Coetzer et al. 2001).

*Glufosinate efficacy on weeds of interest.* Glufosinate has been shown to have differential efficacy among weed species. Common lambsquarters (*Chenopodium album* L. ) has been shown to have a low level of tolerance to glufosinate (Steckel et al. 1997c). Other research has shown that glufosinate at similar rates is more effective on common lambsquarters than glyphosate (Higgins et al. 1991; Tharp et al. 1999). Giant foxtail requires less than half the rate of glufosinate to achieve 50% control as compared to velvetleaf or common lambsquarters (Steckel et al. 1997b). Velvetleaf and giant foxtail control were 100% from one application at the 400 g/ha rate, while more was required for control of common lambsquarters (Krausz et al. 1999). Glufosinate has low long term efficacy on perennial weeds like Canada thistle due to the small amount of translocation causing regrowth from the roots (Pline et al. 2000) Glufosinate resistance is rumored to have been reported on two species; rigid rye grass (*Lolium rigidum* Gaudin.) and goosegrass (*Eleusine indica* L. Gaertn.), although not yet published (Ian Heap, personal communication, October 13, 2009).

**Chlorimuron-ethyl.** Chlorimuron-ethyl (2-[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] benzoic acid) is a selective



sulfonylurea herbicide used preemergence and postemergence primarily for broadleaf weed control in soybean (Claus 1987). Chlorimuron was introduced in 1986 as CLASSIC® by DuPont, the appeal of chlorimuron was its control of broadleaf weeds at significantly low application rates and the tolerance that soybean exhibits to the herbicide (Claus 1987). Chlorimuron inhibits acetolactate synthase (ALS), inhibiting the branched chain amino acids, valine and isoleucine (La Rossa and Schloss 1984). Soybean is tolerant to chlorimuron and can be applied over the top at labeled rates with minimal crop injury (Krausz et al. 1992). Chlorimuron also exhibits residual weed control which could be beneficial for longer term control in the field. It persists longer in soils with high pH and has an average field half-life of 40 days (Wauchope 1992).

Susceptible weeds show rapid inhibition of growth when chlorimuron is applied (Claus 1987). Chlorosis is observable to the naked eye 3 to 5 days after application followed by necrosis of the apical meristem. Complete control is exhibited within 7 to 21 days on susceptible plants. Some species will never exhibit the necrosis and will remain green but stunted. Chlorimuron is readily absorbed in sensitive species and translocated to the meristems of roots and shoots (Claus 1987).

*Chlorimuron efficacy on weeds of interest.* Control of velvetleaf by the full rate of chlorimuron is 75% (Claus 1987). Chlorimuron has very little efficacy on common lambsquarters with over 50 g/ha required for complete control (Lich et al. 1997). Chlorimuron does not control grass species (Jordan et al. 1997). Control of Canada thistle by chlorimuron was 43% when the adjuvants crop oil

concentrate (COC) and 28% urea ammonium nitrate (UAN) were used, control was increased to 77% when non-ionic surfactant (NIS) was used (Sprague et al. 1999).

**Thifensulfuron-methyl.** Thifensulfuron-methyl (methyl 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate), a sulfonylurea, is an ALS inhibitor, applied postemergence. It was first introduced and sold as PINNACLE by Du Pont in 1987 for use in wheat and soybean.

Symptoms in sensitive plants are similar to those exhibited with chlorimuron injury, growth is inhibited within hours of application while injury symptoms appear 1-2 wk later (Sensemen 2007). It is readily absorbed by foliage and roots and is translocated through the xylem and phloem with accumulation in meristematic areas. Its persistence in the soil can be up to 6 d under aerobic conditions and 28 d in anaerobic conditions.

*Thifensulfuron efficacy on weeds of interest.* Thifensulfuron generally has good efficacy on velvetleaf and common lambsquarters when applied at labeled rates with adjuvants (Fielding and Stoller 1990). Research on thifensulfuron activity in Canada thistle and giant foxtail has not completed to date.

**Adjuvants.** The addition of ammonium sulfate (AMS) increases the control of many weeds with weakly acidic herbicides such as glufosinate (Maschhoff et al. 2000; Pline et al. 2000; Young et al. 2003); and glyphosate (Donald 1988; Salisbury 1991; Turner and Loader 1975; Wills and McWhorter 1985).

The addition of AMS to glufosinate has been shown to increase the uptake by 23% (Maschoff et al. 2000). However, the addition of AMS to glufosinate has also been reported to decrease glufosinate absorption in common lambsquarters (Pline et al. 1999). The addition of AMS to glyphosate failed to increase glyphosate efficacy on common lambsquarters (Young et al. 2003). Ammonium sulfate addition increased absorption translocation of the herbicides in the plant.. The addition of AMS to glyphosate has been shown by numerous studies to be advantageous, with a two-fold increase in glyphosate rate required to meet the equivalent control of the addition of AMS (Young et al. 2003). Glyphosate benefits more from the addition of AMS when applied at lower rates than at higher rates (Young et al. 2003).

### **TANK-MIX INTERACTIONS OF HERBICIDES**

Combining herbicides is a tool producers can use to increase the range of control. By combining herbicides with different modes of action and different target weeds, a farmer can potentially increase the spectrum of weed control in his field with less application cost. Tank-mixtures, much like herbicide rotation, are also used to prevent the development of herbicide resistant weeds by altering selection pressures. Tank-mixing has been shown to be more effective in reducing resistance evolution than using herbicides in a rotation (Hugh and Reboud 2009). Much research and review has been done on the joint action of herbicides in a tank-mix (Colby 1967; Hatzios and Penner 1985; Streibig et al. 1998). Information on the potential effects of reduced rate dosage due to environmental factors like rain could be determined from studying the joint action

of herbicides at a range of rates (Streibig et al. 1998). Common terminology used to report results of combinations and interactions are; synergistic, antagonistic and additive (Morse 1978). There are many differential definitions for these terms used across the science disciplines. Synergism is defined as “The combined action of two or more agents that is greater than the sum of the action (sic) of one of the agents used alone.” (Anonymous 2010). Antagonism is defined as the interaction between two or more chemical substances that diminishes the effect that each of them has individually (Anonymous 1989). These definitions are similar to those found in the herbicide handbook and are the official definitions of the WSSA (Sensement 2007). Much controversy is raised around the issue classifying a combination as synergistic or antagonistic. This is due to the contrasting definitions and the confusion of the word sum in the definition of synergism (Morse 1978).

A large number of herbicide combinations have resulted in herbicide antagonism (Hatzios and Penner 1985; Zhang et al. 1995). The mechanisms of herbicide antagonism are classified as being either biochemical, competitive, physiological or chemical (Morse 1978). Biochemical antagonism occurs when the antagonist interacts with the other herbicide to decrease the amount available at the site of action, usually the result of reduced herbicide absorption, altered transport and increased biotransformation (Hatzios and Penner 1985). Competitive antagonism occurs when herbicides with the same site of action are applied and is a function of the concentration as well as affinity for site of action (Hatzios and Penner 1985). Physiological antagonism occurs when two

herbicides affect two different modes of action that produce opposite effects of the physiological process (Hatzios and Penner 1985). Chemical antagonism occurs when the two herbicides mixed interact in the mixture to form some type of inactive chemical complex (Hatzios and Penner 1985).

As different weed species react differently to the application of one herbicide, they also react differently to a tank-mixed herbicide. A review by Zhang et al. (1995) found there was little difference in the amount of synergism and antagonism in monocot species but that there were significantly more results of antagonism on monocot species. Differences in injury were also seen between weed families. In 12 studies on the Chenopodiaceae family only one resulted in a synergistic response. The Compositae, Gramineae and Leguminosae families showed more antagonistic interactions than synergistic. Zhang inferred that the genetic, physiological, morphological and biological differences between species and families are what may be responsible for the differential response.

Tank mix interactions can occur between selective herbicides such as the ALS inhibitors in this study and non-selective herbicides such as glyphosate and glufosinate. It is a common practice to mix slow acting systemic and fast acting burn down herbicides for a broad spectrum of control (Green 1989). The most commonly seen interaction between these herbicides is antagonism, especially at reduced rates (Hydrick and Shaw 1994). Research has indicated that with an increase in the rate of the non-selective herbicide, antagonism can be overcome while still keeping the rate of the selective herbicide (Hydrick and Shaw 1994; Rhodes Jr. and Coble 1984). A rate response has been observed with many tank

mix interactions. One example was that increasing a glyphosate rate in combination with acifluorfen eliminated antagonism (Jordan et al. 1997). Reduced rates of many herbicides can still have the same efficacy as the full recommended rate and using these rates in combination with other herbicides is a common practice (Green 1991).

**Tank-mixing glyphosate and the sulfonylureas.** The Herbicide Handbook states that chlorimuron may have reduced efficacy when tank-mixing with other herbicides (Sensemen 2007). One study found that chlorimuron and glyphosate were either additive or antagonistic depending on rates applied and species applied to (Starke and Oliver 1998). A recent study found that glyphosate tank-mixed with one-half label rates of chlorimuron gave the highest percent control (80%) of the combinations studied on common lambsquarters (Knezevic et al. 2009). The addition of chlorimuron to glyphosate did not reduce the efficacy of glyphosate in velvetleaf (Jordan et al. 1997). Variation between years was found in 1996 and 1997 field studies. In 1996 Vidrine et al. 2002 found that at all rates of chlorimuron plus glyphosate had greater control than glyphosate applied alone, however this was not observed in 1997 (Vidrine et al. 2002). These studies were not subjected to Colby's analysis of interaction and thus mixed results could have been observed. Tank-mixing glyphosate plus chlorimuron and thifensulfuron resulted in lower soybean yields compared with glyphosate alone but with no significant differences in weed control (Corrigan and Harven 2000).

**Tank-mixing glufosinate and the sulfonylureas.** Research on tank mixtures of glufosinate and the sulfonylureas is limited to a few studies. Glufosinate combined with chlorimuron and metribuzin resulted in an additive effect in sicklepod (*Senna obtusifolia* L. H.S. Iriwn & Barneby ), antagonism at low glufosinate rates in entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula*) and antagonism at high glufosinate rates in johnsongrass (*Sorghum halepense* L. Pers.) (Hydrick and Shaw 1994, 1995). The authors determined that the addition of a selective foliar herbicide to the non-selective glufosinate normally resulted in antagonism but could usually be overcome when glufosinate was applied at higher rates. As the results show this was not always true but was true for the broadleaf weeds studied. No known studies have completed looking at the interaction between glufosinate and thifensulfuron.

**Tank-mixing glyphosate and glufosinate.** Recent reports have documented the interaction between glyphosate and glufosinate (Chuah et al. 2008; Everman et al. 2009; Kudsk and Mathiassen 2004; Whitaker 2010). One study observed antagonism between the two herbicides in goosegrass (*Eleusine indica*) at all rates (Chuah et al. 2008). They attributed this observed antagonism to the fast acting nature of glufosinate injuring the plant before the slower acting systemic glyphosate can act (Chuah et al. 2008). Another study that observed antagonism between glyphosate and glufosinate also observed synergism (Kudsk and Mathiassen 2004). Although little research to date has been published on the interaction of glyphosate and glufosinate, antagonism has been reported

between glyphosate and other contact herbicides (Appleby and Somabhi 1978; Hayward et al. 1988; Hydrick and Shaw 1994; Lich et al. 1997; Wehtje et al. 2008). Glufosinate acts faster than glyphosate to injure the plant, much like diquat, a bipyridilum contact herbicide. In diquat plus glyphosate treatments, early synergism was observed between the chemicals (4 DAT), but later antagonism was observed due to increased regrowth (Wehtje et al. 2008). Higher glyphosate rates were needed to compensate for the inhibition of glyphosate activity caused by the rapid plant death and retention of glyphosate in the treated leaf. An example of synergism of fast acting herbicides is diuron and paraquat. Diuron quickly inhibits photosynthesis before paraquat can cause cell destruction and allows limited paraquat translocation to unsprayed portions of the plant (Hayward et al. 1988).

**Evaluation of the interactions.** Interactions could be deemed antagonistic, synergistic or an additive effect based upon the statistical methodologies used to compare them. Two models are commonly used to determine the joint action of herbicides; the Additive Dose Model (ADM) and the Multiplicative Survival Model (MSM) (Morse 1978; Streibig et al. 1998). The ADM is used when looking at the joint action of herbicides of the same mode of action and assumes that the effect of each herbicide dose could interchange with each other and not affect efficacy (Streibig et al. 1998). The MSM assumes that each herbicide has a different mode of action and acts independently on the plant resulting in a multiplicative effect (Streibig et al. 1998). The fundamental difference is that the MSM



considers effects while ADM considers dose rates. MSM is the most commonly used method in weed science in the form of Colby's equation (Colby 1967), which does not require estimation of dose-response curves (Morse 1978). There is no agreement on which of these models is the best to use, however it has been argued that conceptually ADM has more advantages than MSM and so MSM should only be used when looking at mixtures of herbicides with different modes of action (Streibig and Jensen 2000). It is for that reason that the MSM was used for this research.

## **ABSORPTION AND TRANSLOCATION OF HERBICIDES**

Absorption and translocation of herbicides varies dependent upon the herbicide applied, weed species and the environment. For herbicides to be effective they must come into contact with the living tissue. The effectiveness of the herbicide is directly related to its absorption in the plant and for systemic herbicides, movement throughout the plant. The reason for the interaction between herbicides can be observed through absorption and translocation studies. The interaction between glyphosate and glufosinate has been studied through absorption and translocation studies at MSU and resulted in antagonism of the two herbicides on giant foxtail due to reduced absorption and translocation out of the treated leaf (Everman et al. 2009).

**<sup>14</sup>C-glyphosate.** Glyphosate is absorbed across the cuticle when applied postemergence (Boerboom and Wyse 1988). Glyphosate is a non-polar herbicide

and of the non-polar herbicides has the slowest transport across plasmalemma (Jachetta et al. 1986)

Translocation of glyphosate is primarily in the symplast with accumulation of glyphosate occurring in the immature leaves, meristems and underground tissues (Martin and Edgington 1981). There is little evidence of apoplastic movement but it has been observed (Sprankle et al. 1975).

Absorption of  $^{14}\text{C}$ -glyphosate increases over time. Foliar absorption of glyphosate commonly ranges between 25 and 50% (Sprankle et al. 1975; Wyrill and Burnside 1976; Young et al. 2003). Sandberg et al. (1980) reported that glyphosate translocation was 3 to 20% of  $^{14}\text{C}$  applied at 72HAT in five species. Translocation of  $^{14}\text{C}$  glyphosate to the roots of Canada thistle was greater when applied at the rosette than the bud-stage (Hunter 1996).

Growth stage and plant stress before herbicide application reduce the absorption and translocation of glyphosate in many species (Ahmadi et al. 1980, Pline et al. 2001). The addition of adjuvants increases absorption which increases overall translocation throughout the plant (Sherrick et al. 1986, Sprankle et al. 1975).

**$^{14}\text{C}$ -chlorimuron.** Chlorimuron is a systemic herbicide that is readily taken up by both roots and shoots and is translocated to the meristematic regions (Claus 1987). Adding glyphosate to the tank mix increased the absorption of  $^{14}\text{C}$ -chlorimuron in Palmer amaranth and velvetleaf, this increase was attributed to the addition of AMS when glyphosate was added (Starke and Oliver 1998).

However, translocation by sulfonyleureas out of the treated leaf is generally limited. Chlorimuron absorption was less than 53% after 96 hours and translocation was 30% out of the treated leaf in yellow nutsedge (*Cyperus esculentus* L.). Only 4% of the translocated herbicide was found in the underground tubers (Troxler et al. 2003).

The objective of these studies was to evaluate the interaction between glyphosate, glufosinate and the sulfonyleurea herbicides chlorimuron and thifensulfuron in a matrix combination of all rates studied. By determining if any interactions exist we can conclude that herbicide programs in stacked gene traits and field with herbicide resistant weeds need to be adjusted. To determine the basis of the interaction we will look at absorption and translocation of glyphosate and chlorimuron alone and in combination and will use fluorescence measurements to determine how quickly the interaction takes place before it is visually observable.

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## CHAPTER 2

### EARLY DETECTION OF HERBICIDE INTERACTIONS WITH FLUORESCENCE MEASUREMENTS

**Abstract:** Tank-mixing of herbicides with different chemistries provides the opportunity to control a wider range of weed species and herbicide resistant weeds. Interactions between glyphosate, glufosinate, and chlorimuron have been observed in the greenhouse and the field. The objective of this study was to evaluate the combinations of glyphosate, glufosinate and chlorimuron on three annual weeds; giant foxtail, common lambsquarters, and velvetleaf and the perennial weed, Canada thistle using fluorescence. Fast acting herbicides like glufosinate cause rapid inhibition of photosynthesis which is observable through fluorescence measurements before injury may be visible. When applied alone or in combination with these herbicides, glufosinate caused a rapid decrease in the maximum capacity for photochemical quenching evident by the parameters derived from the OJIP curve;  $F_v/F_m$  (maximum quantum efficiency of photosystem II),  $F_vj$  (relative changes in the J step) and changes in the Kautsky/OJIP curve. Changes in  $F_v/F_m$  were observable within 2 hours after treatment (HAT) when glufosinate was applied alone or in combination, indicative of the rapid breakdown of the plants protective non-photochemical photosynthetic systems. Changes in  $F_v/F_m$  were observable within 24 HAT with glyphosate applied alone and in combination with chlorimuron, whereas application of chlorimuron alone produced no observable changes until 72 HAT. The fast action of glufosinate on the photosynthetic system may limit translocation and expression of the activity of glyphosate and chlorimuron and results indicate that

the combinations of glufosinate with glyphosate and chlorimuron can be antagonistic.

**Nomenclature:** Canada thistle (*Cirsium arvense*), chlorimuron, common lambsquarters (*Chenopodium album*), giant foxtail (*Setaria faberi*), glufosinate, glyphosate.

**Key words:** fluorescence, Fv/Fm, Kautsky induction curve, OJIP curve

Early detection of herbicide injury is an important means for detection of misapplication in the field and for research on herbicide mode of action. Early detection refers to using other modes of determining plant response to herbicide injury prior to the observation of visual injury. Early detection of herbicide injury by fluorescence measurements has been used successfully in many studies (Barbagallo et al., 2003).

Glyphosate, glufosinate, and the sulfonylurea herbicides, although not primarily Photosystem II (PS II) inhibitors, do ultimately cause cellular death resulting in a weakened ability to use or disperse light energy. Changes in fluorescence induction (Kautsky curve) have been used extensively in photosynthesis and herbicide research and are the basis for all fluorescence parameters (Abbaspoor and Streibig, 2005; Christensen et al., 2003; Percival and Baker, 1991). The benefits of using fluorescence include its non-invasive procedure, sensitivity to many biotic and abiotic stressors, ease and efficiency and numerous parameters to measure the status of the photosynthetic apparatus (Abbaspoor and Streibig, 2005; Barbagallo et al., 2003; Frankart et al., 2003; Strasser et al., 2000).



Several parameters are used to measure the fluorescence signal. The most important are those parameters that can be used to elucidate differences in changes to the PS II system. Illumination of dark-adapted unstressed leaves produces a rise in chlorophyll fluorescence emission from the ground state ( $F_0$ ) to its maximum value ( $F_m$ ) within one second (Figure 1). Within that second other parameters are also observed. One such parameter is the JIP-test. Based on the Kautsky curve the polyphasic rise of OJIP is observed in all plant species. The shape of this curve is very sensitive to stressors (Percival and Baker, 1991; Strasser et al., 2000). OJIP has three distinct phases associated with events in PSII (Govindjee, 1995). The three phases are as follows: (1) (O to J) phase corresponds with the complete reduction of  $Q_A$  of PS II, (2) (J to I) phase corresponds to the transfer of electrons, from  $Q_A$  to  $Q_B$ , this phase is controlled by the PS II donor side which is responsible for water splitting activity, (3) (I to P) phase which corresponds to the release of fluorescence quenching by the oxidized plastoquinone pool (Figure 1). The J to I phase is of particular interest because it is an indicator of the water-splitting activity of PS II. The relative changes at the J step [ $F_vj = (F_m - F_j)/F_m$ ] can be indicative of a reduction of the activity of PS II. Reductions in the rise of these phases correspond to the destruction of the particular photochemical mechanism, therefore it may be possible to pin-point exactly when and where these non PS II mode of action inhibitory herbicides act on the PS II system by looking at where the curve flattens.

Another important parameter is the Fv/Fm [ $Fv/Fm = (Fm - Fo)/Fm$ ] (Figure 1) parameter (Butler, 1978). The dark adaptation of a leaf allows PSII to be fully reduced at  $Q_A$  and when illuminated the maximum quantum efficiency of the PS II photochemistry can be determined by Fv/Fm. This parameter is used most often in the literature to indicate plant health with a value of 0.83 indicating no stress to the plant. Fv/Fm has been used to measure the effect of glyphosate on fluorescence in previous studies. Kirkwood et al. 2000 used this parameter and detected some differences from the control one day after treatment while neither Olesen and Cedergreen, 2010 or Ralph 2000 found any effect of glyphosate on Fv/Fm. Changes in the Fvj parameter derived from Kautsky curve were observed 4 hours after glyphosate application when applied on sugar beet (*Beta vulgaris* L.) and at 24 hours after application in white mustard (*Sinapis alba* L.) (Christensen et al. 2003).

The objectives of this study were to determine if fluorescence measurements of herbicide treated plants were different among species and determine if there were fluorescence differences among herbicide modes of action. We hypothesize that there will be differences in how species respond to herbicides indicated by fluorescence parameters. Secondly, there will be mode of action differences in fluorescence parameters, that the different parameters; Fv/Fm, Fvj and OJIP curves will show similar to herbicide applications, and fluorescence parameters will indicate injury to the photosynthetic system much earlier than visually observed.

## MATERIALS AND METHODS

**Plant material.** Common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.) and giant foxtail (*Setaria faberi* Herm.) were grown from seed in the greenhouse. Approximately 10 seeds each were sown into soil media<sup>a</sup> in 900 mL black plastic pots. Plants were thinned to one per pot upon emergence. Canada thistle (*Cirsium arvense* L. Scop.) plants were grown from root stock<sup>b</sup> obtained in May of 2008 and transplanted into soil media in 900 mL black plastic pots. These plants were genetically similar. Tillers from stock plants were transplanted into fresh media and pots. Canada thistle plants were selected for treatment 2 weeks after transplant.

All plants were grown in greenhouses at Michigan State University campus in East Lansing, MI and experiments took place in May of 2009. Natural light was supplemented by high-pressure sodium lamps that produced a photosynthetic photon flux density of  $200 \text{ mol m}^{-2} \text{ s}^{-1}$ . The photoperiod was 16/8 h light/dark, and the temperature was  $23 \pm 3^\circ\text{C}$ . Plants were fertilized with 50 ml of fertilizer solution containing 6 mg/L of 20% nitrogen, 20%  $\text{P}_2\text{O}_5$  and 20%  $\text{K}_2\text{O}$  as needed. Plants were 10-12 cm tall at time of treatment and were randomly assigned to herbicide treatments. Treatments were replicated three times and the experiment repeated three times with common lambsquarters and Canada thistle. Treatments were replicated three times and the experiment conducted one time with velvetleaf and giant foxtail.

**Herbicide treatments.** Herbicide treatments consisted of glyphosate, glufosinate and chlorimuron alone and in combinations at the rates seen in Table 1. Ammonium sulfate (AMS) at 2% v/v was used as an adjuvant when glyphosate or glufosinate were applied alone or in combination with chlorimuron. Crop oil concentrate was used at 1% v/v when chlorimuron was used alone. Treatments were applied using a single-tip track sprayer using a TP8001 flat fan nozzle<sup>c</sup> delivering 187 L ha<sup>-1</sup> at a pressure of 207 kPA. Treatments were based on preliminary studies which showed these rates had the highest observable interaction and were also the most economically interesting, such as, a high and a low rate combined, low rates combined and high rates combined.

**Fluorescence measurements.** After herbicide application the plants were immediately returned to the greenhouse and prepared for fluorescence reading. Fluorescence readings were taken at 2, 4, 6, 8, 24, 48 and 72 hours after treatment (HAT). The second set of fully emerged leaves above the cotyledons, with at least one more set of fully emerged leaves above were selected for fluorescence evaluation. Leafclips<sup>d</sup> were placed in the middle of the selected leaf directly next to the midvein with the least amount of contact with any major veins. The clip has a small shutter plate that must be closed over the leaf once the clip is attached so that light is excluded and dark adaptation begins to take place. The process of dark adaptation varied depending on plant species, ambient light history and whether the plant was stressed. The average time required for dark adaptation in this study was 15 minutes. Once dark adapted, the Handy Pocket PEA<sup>e</sup> optical interface is attached to the clip around the shutter plate, the shutter

was opened and high intensity LED light passed through a NIR filter, onto the leaf. Then a highly sensitive PIN photodiode detects the fluorescence signal at 10 $\mu$ s intervals for 1 second. The data obtained in the 1 second period was saved in the Handy Pocket PEA and later downloaded into a computer. The Kautsky curves for different doses and time intervals were visually examined to determine the effect of time and dose. Parameters from the Kautsky curve were obtained using the Handy Pocket PEA software. Data were subjected to ANOVA using PROC MIXED in SAS<sup>f</sup> and treatment means for Fv/Fm and Fvj with species were compared using Fisher's Protected LSD at the  $p = 0.05$  significance level. Among species comparisons were done by creating contrast statements analyzing differences between species with a chosen LSD = 0.1.

## RESULTS AND DISCUSSION

**Species differences.** Data from the application of the 1X rate of each mode of action on all four species was analyzed to test the hypothesis that there would be differences in response to herbicides among species (Figure 2 and 3). Multiple parameters were chosen for comparison; Fv/Fm and Fvj characterizes species response to herbicide application. The Fv/Fm of a healthy plant is 0.83, a value smaller than 0.83 is an indication of the inability of the plant, due to injury, to efficiently photosynthesize. Fvj is indicative of the flattening of the OJIP curve at the J to I phase. Both a lower Fvj and the flattening of the OJIP curve at the J to I phase indicates that the plant is unable to effectively transfer electrons from QA

to  $Q_B$ . Observed flattening of the OJIP curve at the other phases can indicate stress on other photosynthetic processes outlined in the introduction.

The following are observations based on the data obtained from the fluorescence experiments. The  $F_v/F_m$  at 2 HAT of the 1X glyphosate rate on Canada thistle were significantly higher than all other species except giant foxtail. The  $F_v/F_m$  values for glyphosate at 48 HAT were lowest in Canada thistle and highest in giant foxtail though not significantly different (Figure 2a).  $F_vj$  values for the glyphosate treatment followed the same trend as  $F_v/F_m$  (Figure 3a). There were no species differences following the 1X glufosinate treatment at 2 HAT, but at 4 HAT  $F_v/F_m$  of giant foxtail were significantly lower than for common lambsquarters.  $F_v/F_m$  values for glufosinate treatments were lowest in Canada thistle and highest in giant foxtail at 48 HAT (Figure 2b).  $F_vj$  values for glufosinate treatments responded similarly as  $F_v/F_m$  but at 48 HAT  $F_vj$  were highest for common lambsquarters (Figure 3b). The  $F_v/F_m$  following the 1X chlorimuron applications to Canada thistle were significantly higher than for all other species at 2 HAT. The  $F_v/F_m$  for common lambsquarters had the lowest  $F_v/F_m$  at this time (Figure 2c). The  $F_v/F_m$  for giant foxtail at 48 HAT was higher than all other species. The  $F_v/F_m$  for Canada thistle was significantly lower than common lambsquarters at 72 HAT. The  $F_vj$  for chlorimuron showed less significant differences but were still apparent (Figure 3c). The  $F_vj$  and  $F_v/F_m$  showed similar trends for differences among species. Induction curves showed that there were differences among species OJIP readings, and  $F_o$  and  $F_m$  readings at 48 HAT (Figure 4). Following glyphosate application the OJIP curve

for common lambsquarters was the most like the normal OJIP curve while the I peak was no longer observable in any species (Figure 2, 4a). Glufosinate application resulted in a larger reduction in Fv/Fm observable by the extreme flattening of the OJIP curve in all species (Figure 4b). The curve for common lambsquarters was the only one to still show the P peak while the curve for Canada thistle showed no peaks. Chlorimuron application resulted in less changes in the OJIP curve than was observed for the other herbicides but the I peak in the curve was still not observable (Figure 4c). The OJIP curve for common lambsquarters differed the most from the others and was the most similar to the curve for the control (Figure 1, 4c).

**Mode of action.** The mode of action differences for the Fv/Fm were compared for the 1X rate of each herbicide applied alone. Analysis of Fv/Fm data facilitated comparison of species differences between Canada thistle and common lambsquarters (Figure 5). Mode of action differences were observable for Fv/Fm values of Canada thistle at 2 HAT. Fv/Fm following application of 1X glufosinate was significantly lower than the control from 2 HAT to 72 HAT (Figure 5a). The Fv/Fm for plants receiving application of 1X glyphosate also were significantly lower than the control at 24 HAT. The Fv/Fm for those sprayed with glufosinate were always significantly lower than Fv/Fm for glyphosate except at the 72 HAT reading (Figure 5a). Fv/Fm values for Canada thistle that were sprayed with chlorimuron were never significantly different from the control. Mode of action differences in Fv/Fm values among herbicides were observed in common lambsquarters beginning at 6 HAT. The Fv/Fm values following application of 1X

glufosinate were significantly different from the control and other herbicides at 6 HAT (Figure 5b). Fv/Fm values after glufosinate application remained significantly different from the control and other herbicide treatments until 48 HAT. Fv/Fm values at 48 HAT following glufosinate application at 1X were no different from those following glyphosate at 1X but both were significantly lower than the control and following the 1X chlorimuron application (Figure 5b). Fv/Fm values at 72 HAT after glufosinate application were significantly lower than following glyphosate application however; they are both significantly different from the control and following chlorimuron application. Fv/Fm values for common lambsquarters receiving chlorimuron were not significantly different from the control during the course of observation (Figure 5b). OJIP curves at 48 HAT showed differences between species and herbicide mode of action (Figure 6). Canada thistle had lower Fm values than common lambsquarters regardless of herbicide modes of action (Figure 6). Glufosinate application to Canada thistle caused a complete collapse of all OJIP peaks whereas for common lambsquarters this curve still had the P peak. In Canada thistle the collapse of the glyphosate curve was more obvious than in common lambsquarters (Figure 6). Plants receiving chlorimuron treatments responded similarly to the control for both species (Figure 6).

This study showed that there were differences in fluorescence values within species and from herbicides with different modes of action. Species differences were characterized with Fv/Fm and Fvj parameters. These parameters presented similar data trends to one another. Comparison of OJIP



curves among species showed species dependent responses to herbicide modes of action. Modes of action differences for herbicides were evident with fluorescence patterns like Fv/Fm. This parameter showed glufosinate action rapidly (2 HAT) resulted in a breakdown of the photosynthetic apparatus. OJIP curves of all species responded to glufosinate application with complete flattening of all phases except the I to P phase in common lambsquarters. Glyphosate application resulted in a flattening of the OJIP curve and a reduction in Fv/Fm beginning at 24 HAT. Whereas chlorimuron application did not result in flattening the OJIP curve. Thus, fluorescence measurements indicated injury to the photosynthetic system much earlier than was visually observable. With glufosinate we saw reductions in fluorescence parameters evident at 2 HAT following glufosinate application and at 24 HAT with glyphosate. Visible injury was not viewable until 3 to 5 days after treatment and 7 to 10 days after treatment, respectively.

## **SOURCE OF MATERIALS**

<sup>a</sup>Baccto® High Porosity Professional Potting Mix, Michigan Peat Co., Houston, TX.

<sup>b</sup>Don Penner's Farm Williamston, MI. pennerd@msu.edu.

<sup>c</sup>TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

<sup>d</sup>Leaf Clips, Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn, Norfolk, U.K.

<sup>e</sup>Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn Norfolk, U.K.

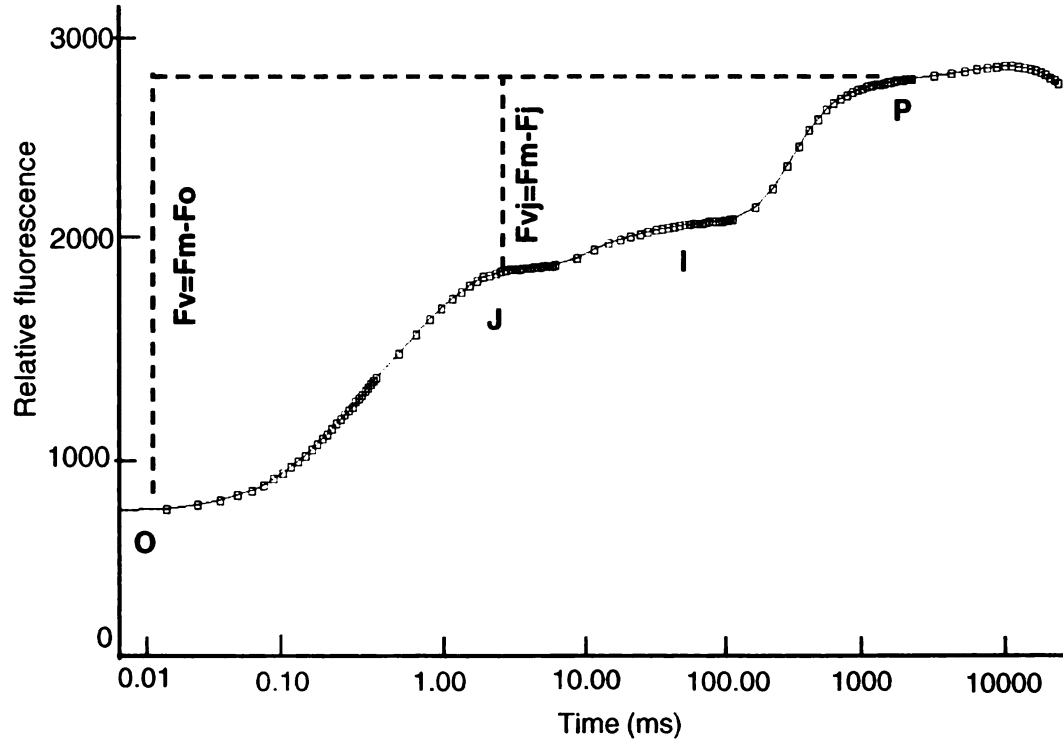
<sup>f</sup>The SAS System for Windows, Version 9.2, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513.

**Table 1.** Herbicide combinations applied in fluorescence studies.<sup>a</sup>

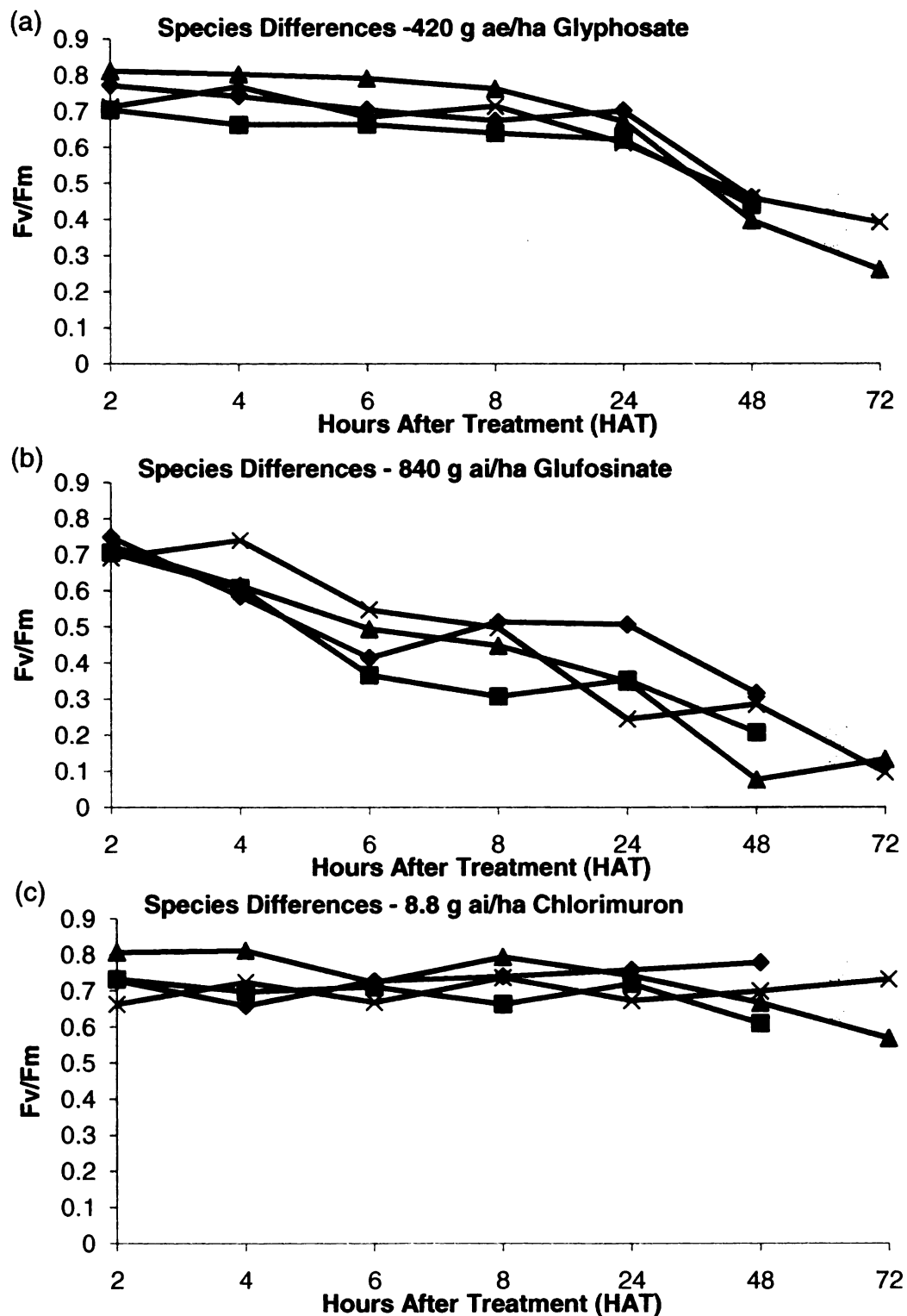
<b>Herbicides Applied by Species - Fluorescence Studies</b>	
<b>CIRAR + CHEAL</b>	<b>ABUTH + SETFA</b>
Control	Control
0.25X GLY <sup>b</sup>	0.25X GLY
0.5X GLY	1X GLY
1X GLY	0.25X GLU
0.25X GLU	0.5X GLU
0.5X GLU	1X GLU
1X GLU	1X CHL
0.25X CHL	0.25X GLY + 1X CHL
0.5X CHL	0.25X GLU + 1X CHL
1X CHL	0.25X GLU + 1X GLY
0.25X GLU + 1X GLY	
0.5X GLU + 0.5X GLY	
1X GLU + 0.25X GLY	
0.25X GLU + 0.25X CHL	
0.25X GLU + 1X CHL	
0.5X GLU + 0.5X CHL	
1X GLU + 0.25X CHL	
1X GLU + 0.5X CHL	
1X GLU + 1X CHL	
0.25X GLY + 0.25X CHL	
0.25X GLY + 1X CHL	
0.5X GLY + 0.5X CHL	
1X GLY + 0.25X CHL	
1X GLY + 0.5X CHL	
1X GLY + 1X CHL	

<sup>a</sup> Where 1X = the labeled rate; glyphosate applied as Roundup WeatherMAX 1X = 840 g ae/ha; glufosinate applied as LIBERTY 1X = 420 g ai/ha; chlorimuron applied as CLASSIC 1X = 8.8 g ai/ha.

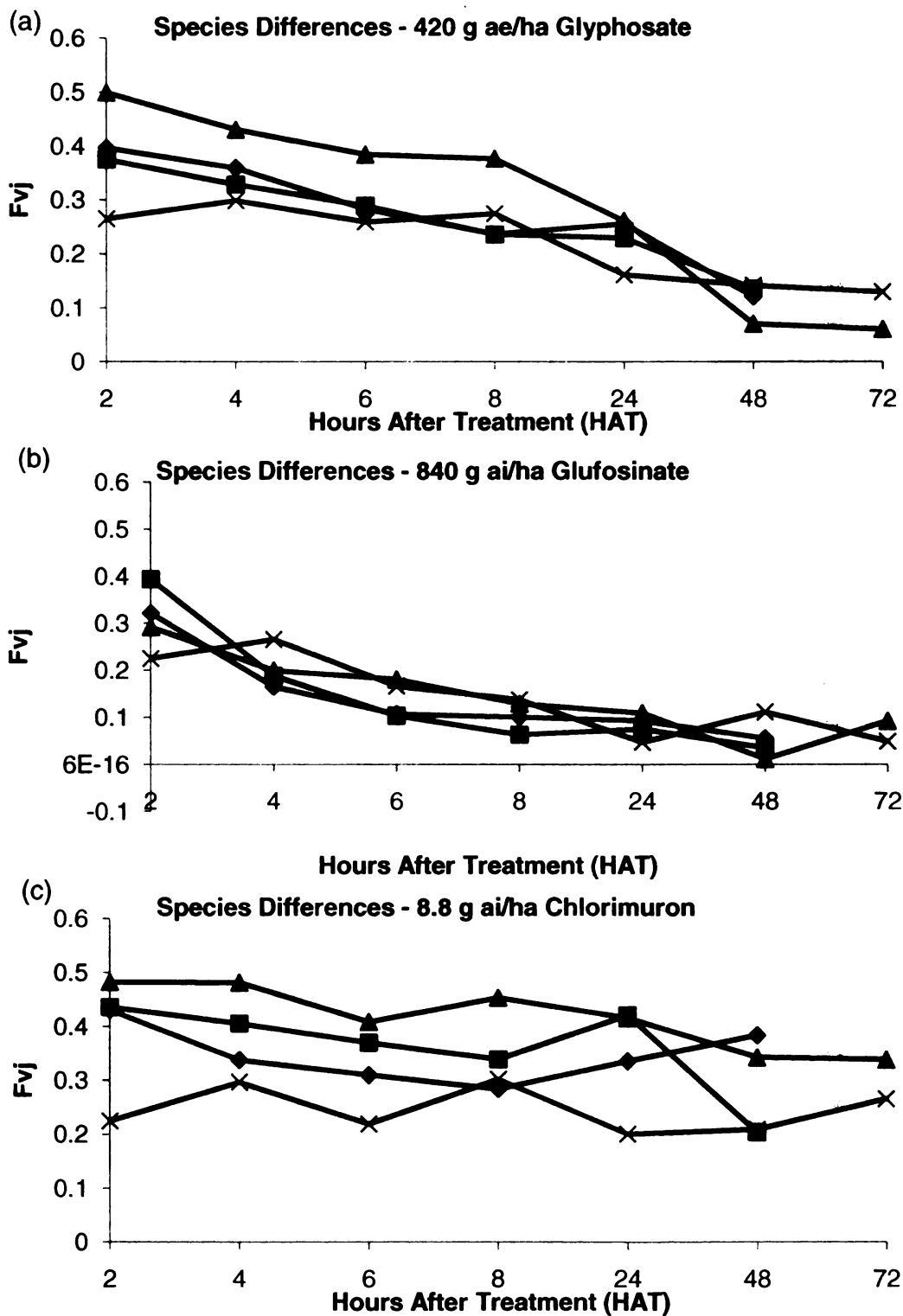
<sup>b</sup> Abbreviations: GLY, glyphosate; GLU, glufosinate; CHL, chlorimuron.



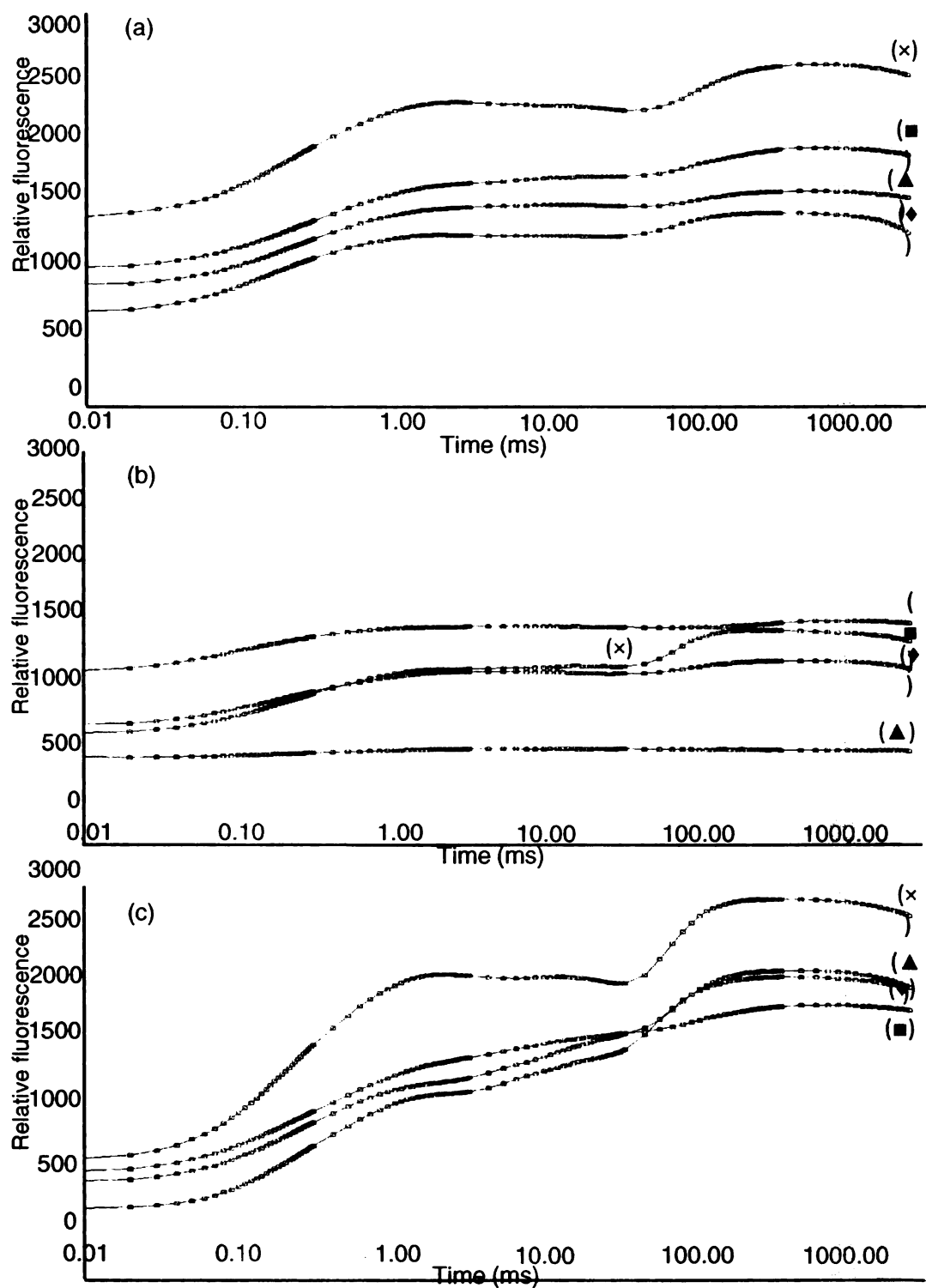
*Figure 1.* Chlorophyll fluorescence induction curve (Kautsky/OJIP curve) of a 15 minute dark adapted healthy Canada thistle leaf.



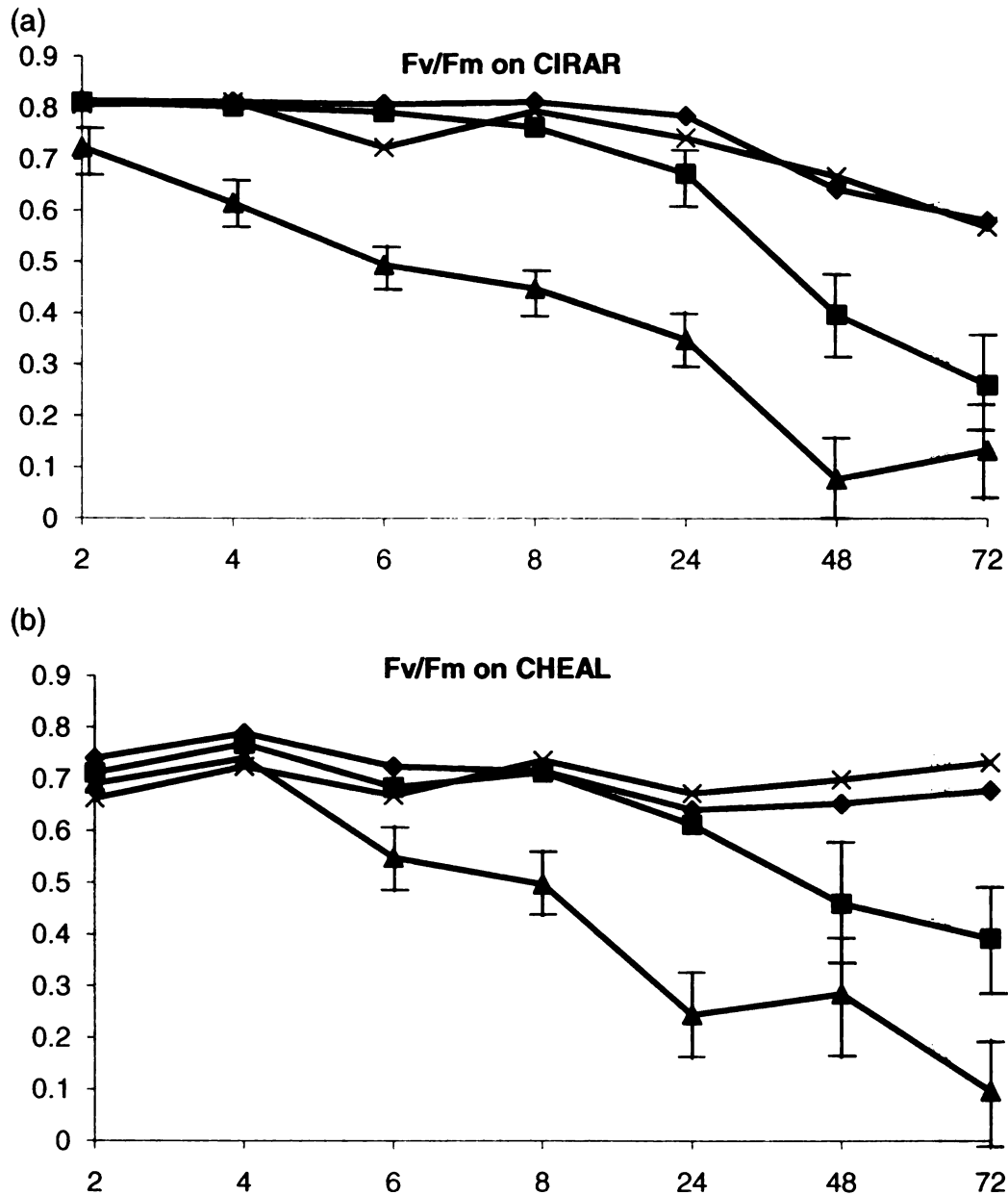
**Figure 2.** Species differences in Fv/Fm by herbicide mode of action (a) glyphosate (b) glufosinate (c) chlorimuron on (◆) giant foxtail, (■) velvetleaf, (▲) Canada thistle, and (×) common lambsquarters



**Figure 3.** Species differences in Fvj by herbicide mode of action (a) glyphosate (b) glufosinate (c) chlorimuron on (♦) giant foxtail, (■) velvetleaf, (▲) Canada thistle, and (x) common lambsquarters

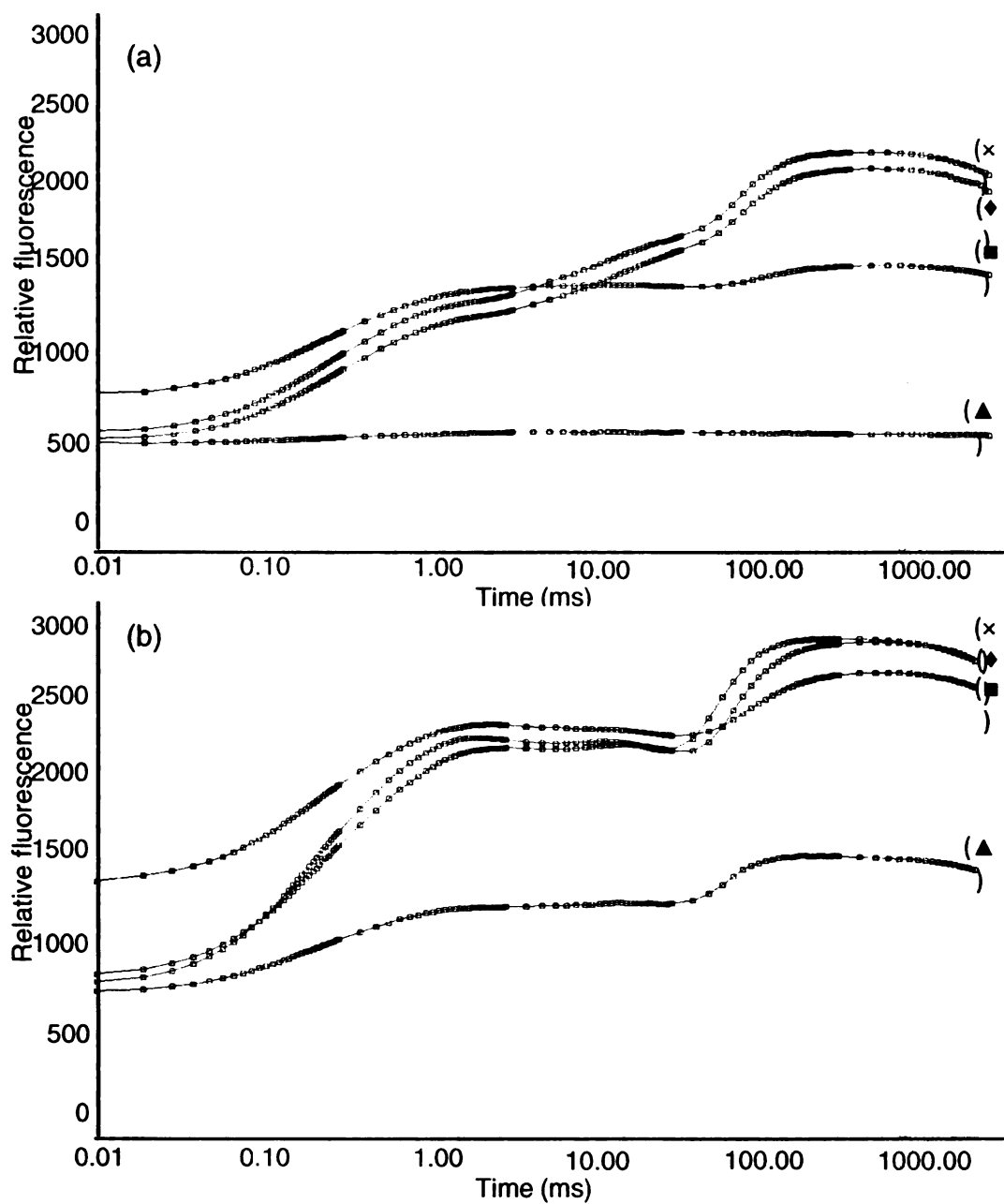


**Figure 4.** Species differences in OJIP curve by herbicide mode of action 48 hours after treatment (a) glyphosate (b) glufosinate (c) chlorimuron on (♦) giant foxtail, (■) velvetleaf, (▲) Canada thistle, and (×) common lambsquarters



**Figure 5.** Fv/Fm differences in mode of action by species (a) Canada thistle (b) common lambsquarters on (◆) control, (■) 1X glyphosate, (▲) 1X glufosinate, and (×) 1X chlorimuron. Vertical bars represent Fisher's protected LSD at p=0.05 significance level, when not present = Not significant.





**Figure 6.** OJIP curve differences in mode of action by species 48 hours after treatment (a) Canada thistle (b) common lambsquarters on (♦) control, (■) 1X glyphosate, (▲) 1X glufosinate, and (x) 1X chlorimuron.

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### CHAPTER 3

## EVALUATION OF THE INTERACTION BETWEEN GLYPHOSATE AND GLUFOSINATE

**Abstract.** The stacking of genes to provide resistance to several herbicides previously injurious to a crop provides new opportunities for control of herbicide resistant weeds. Specifically the opportunity may exist to control glyphosate resistant weeds with glufosinate. The combination of the non-selective foliar herbicides, glyphosate and glufosinate, in a tank mix has the potential to cause unexpected interactions. The objectives of this study were to evaluate the combination of glyphosate and glufosinate on three annual weeds prevalent in Michigan cropping systems; giant foxtail, common lambsquarters, velvetleaf and the perennial weed, Canada thistle and to determine if fluorescence parameters would be indicative of the combined herbicide injury before being visually observable. Field and greenhouse studies showed differential results for the combination of glyphosate and glufosinate on the weed species studied. Early synergism was observed in the greenhouse for giant foxtail and in the field for common lambsquarters, velvetleaf and Canada thistle. This early synergism was not observed in the other species. Field studies to determine herbicide interactions may result in more erratic data due to the effect of environment on herbicide absorption and translocation. Fluorescence measurements in the greenhouse show that although glufosinate does rapidly act to break down the PSII system of photosynthesis, it does affect how the herbicides act in combination on these systems.

**Nomenclature:** Canada thistle, *Cirsium arvense* L. Scop; common lambsquarters, *Chenopodium album* L.; giant foxtail, *Setaria faberi* Herm.; velvetleaf, *Abutilon theophrasti* Medik; glufosinate, 2-amino-4-(hydroxy-methyl-phosphoryl)butanoic acid; glyphosate, N-(phosphonomethyl)-glycine

**Key words:** Additive effect, antagonism, Colby's, herbicide interaction, reduced rates, synergism, tank-mixing.

Combining herbicides is a tool producers can use to increase the range of weed control. By combining herbicides with different modes of action and different target weeds, a farmer can potentially increase the spectrum of weed control in his field with less application cost. Tank-mixtures, much like herbicide rotation, are also used to prevent the development of herbicide resistant weeds by altering selection pressures. Tank-mixing has been shown to be more effective in reducing resistance evolution than using herbicides in a rotation (Hugh and Reboud 2009). However tank-mixing can also result in unexpected interactions between herbicides, such as antagonism.

The stacking of traits for multiple herbicide resistance including those resistant to both glyphosate and glufosinate will give producers the option to apply tank-mixtures of these two broad spectrum, postemergence herbicides potentially allowing for more weed species controlled with one herbicide application.

While studying the interaction of these chemicals it is important to understand their chemistry to determine how they may or may not work together. Glyphosate (N-(phosphonomethyl)-glycine) is an organophosphorus herbicide that inhibits the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSP) stopping the synthesis of the aromatic amino acids, secondary plant metabolites and inhibiting

production of a chlorophyll precursor resulting in reduced chlorophyll synthesis (Amrhein et al. 1980; Jaworski 1972; Kitchen et al. 1981). Glyphosate sensitive weeds exhibit early symptomology of chlorosis followed by necrosis usually from 2 to 10 days after application (Baird et al. 1971).

Glyphosate is a non-selective herbicide; however certain weed species are inherently tolerant to glyphosate. Velvetleaf exhibits a low level of tolerance (Kapusta et al. 1994) whereas the Poacea species like giant foxtail, are susceptible (Sprankle 1974). Common lambsquarters has been shown to have differential susceptibility to glyphosate application, which is thought to be based on growth stage (Jaworski 1972; Lich et al. 1997; Ziska et al. 1999) and others have reported no differences between herbicide responses by growth stage (Sikkema et al. 2004; Tharp et al. 1999). These differences have been attributed to different biotypes at the locations studied at (Schuster et al. 2007). Glyphosate efficacy on Canada thistle is dependent upon the stage of growth, with more injury occurring when controlled in the rosette stage than at bud initiation (Hunter 1995, 1996).

Glufosinate (2-amino-4-(hydroxy-methyl-phosphoryl)butanoic acid) inhibits the production of glutamine synthetase in sensitive species resulting in the buildup of toxic ammonia and an inhibition of photosynthesis causing eventual necrosis of the plant tissue (Hoagland 1983; Lea et al. 1984, Manderscheid and Wild 1986, Ridley 1989, Wild et al. 1987).

Glufosinate sensitive species exhibit symptomology that is similar to that of the PSII inhibitors and only occurs in the presence of light (Hess 2000;

Hoagland 1983). Rate of symptomology after application increases with bright sunlight, high humidity, and moist soil conditions. Under these conditions injury can be seen by 2 days after treatment (Al-Khatib et al. 2003). Glufosinate has been shown to have differential efficacy among weed species. Common lambsquarters has a low level of tolerance to glufosinate (Steckel et al. 1997). Other research has shown that glufosinate at similar rates is more effective on common lambsquarters than glyphosate (Higgins et al. 1991; Tharp et al. 1999). Giant foxtail requires less than half the rate of glufosinate for 50% control than velvetleaf or common lambsquarters (Steckel et al. 1997b). Velvetleaf and giant foxtail control was 100% from one application at the 1X application rate, while more was required for control of common lambsquarters (Krausz et al. 1999). Glufosinate has low long term efficacy on perennial weeds like Canada thistle due to the small amount of translocation causing regrowth from the roots (Pline et al. 2000)

Recent reports have documented the interaction between glyphosate and glufosinate (Chuah et al. 2008; Everman et al. 2009; Kudsk and Mathiassen 2004; Whitaker 2010). Antagonism was observed between the two herbicides in goosegrass (*Eleusine indica* L. Gaertn.) at all studied rates (Chuah et al. 2008). They attributed this observed antagonism to the fast acting nature of glufosinate injuring the plant before the slower acting systemic glyphosate can act (Chuah et al. 2008). Another study that observed antagonism between glyphosate and glufosinate also observed synergism (Kudsk and Mathiassen 2004). Although little research to date has been published on the interaction of glyphosate and

glufosinate, antagonism has been reported between glyphosate and other contact herbicides (Appleby and Somabhi 1978; Hayward et al. 1988; Hydrick and Shaw 1994; Lich et al. 1997; Wehtje et al. 2008). Glufosinate acts faster than glyphosate to injure the plant, much like diquat, a bipyridilum contact herbicide. In diquat plus glyphosate treatments, early synergism was observed between the chemicals (4 DAT), but later antagonism was observed due to increased regrowth (Wehtje et al. 2008). Higher glyphosate rates were needed to compensate for the inhibition of glyphosate activity caused by the rapid plant death and retention of glyphosate in the treated leaf. An example of synergism of fast acting herbicides is diuron and paraquat. Diuron quickly inhibits photosynthesis before paraquat can cause cell destruction and allows limited paraquat translocation to unsprayed portions of the plant (Hayward et al. 1988).

The interaction between glyphosate and glufosinate has been studied through absorption and translocation studies at MSU and resulted in antagonism of the two herbicides on giant foxtail due to reduced absorption and translocation out of the treated leaf (Everman et al. 2009).

Glyphosate and glufosinate, although not primarily Photosystem II (PS II) inhibitors, do ultimately cause cellular death resulting in a weakened ability to use or disperse light energy. Changes in fluorescence induction (Kautsky curve) have been used extensively in photosynthesis and herbicide research and are the basis for all fluorescence parameters (Abbaspoor and Streibig, 2005; Christensen et al., 2003; Percival and Baker, 1991). The benefits of using fluorescence include its non-invasive procedure, sensitivity to many biotic and

abiotic stressors, ease and efficiency and numerous parameters to measure the status of the photosynthetic apparatus (Abbaspoor and Streibig, 2005; Barbagallo et al., 2003; Frankart et al., 2003; Strasser et al., 2000). Illumination of dark-adapted unstressed leaves produces a rise in chlorophyll fluorescence emission from the ground state ( $F_o$ ) to its maximum value ( $F_m$ ) within one second. An important parameter used in fluorescence research is the  $F_v/F_m$  [ $F_v/F_m = (F_m - F_o)/F_m$ ] parameter (Butler, 1978). The dark adaptation of a leaf allows PSII to be fully reduced at  $Q_A$  and when illuminated the maximum quantum efficiency of the PS II photochemistry can be determined by  $F_v/F_m$ . This parameter is used most often in the literature to indicate plant health with a value of 0.83 indicating no stress to the plant.  $F_v/F_m$  has been used to measure the effect of glyphosate on fluorescence in previous studies. Kirkwood et al 2000 used this parameter and detected some differences from the control one day after treatment while neither Olesen and Cedergreen 2010 or Ralph 2000 found any effect of glyphosate on  $F_v/F_m$ .

The objectives of this research were to evaluate potential interactions among four rate matrix combinations of glyphosate and glufosinate in the field and greenhouse, determine if the interactions were significant for antagonism and synergism or just additive, determine if results in the greenhouse were consistent with observations in the field, determine if there were differences between visual control and quantitative measurements, determine if there were different fluorescence responses among species and determine if fluorescence measurements were indicative of early herbicide injury. We hypothesized that



weed control with combinations including low rates of glufosinate and the range of glyphosate will be antagonistic while glufosinate at higher rates will be so fast acting that antagonism will no longer be observed when combined with glyphosate, early synergism between glyphosate and glufosinate would be evident in some species, but by 28 DAT the synergism may no longer be evident and fluorescence measurements would be indicative of the interaction much earlier than visually observed.

## **MATERIALS AND METHODS**

**Field Trial.** Field trials were conducted in 2008 at the Michigan State University Agronomy Research Farm (42°42'42" N, 84°28'13" W) and 2009 at the Michigan State University Plant Pathology Research Center (42°40'59" N, 84°29'5" W) in East Lansing, MI. The soil at the Agronomy Research Farm was a sandy clay loam with 2.6% organic matter and a pH of 6.3. The soil at the Plant Pathology Research Center was a fine sandy loam with a 2 to 6% slope, a pH of 6.9 and 2.5% organic matter. Fields preparation included fall-plowing followed by cultivation in the spring to obtain maximum weed emergence. The experimental design was a randomized complete block in 2008 and 2009. Treatments differed between years and therefore were separated by year and summarized in Table 2.

Herbicide applications were made using a tractor-mounted compressed-air sprayer calibrated to deliver 178 L / ha at 207 kPa through AirMix 11003 nozzles<sup>a</sup>. Common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon*

*theophrasti* Medik.) and giant foxtail (*Setaria faberi* Herrm.) were the predominant weed species in both years and were the focus in this study. Other weed species present included; redroot pigweed (*Amaranthus retroflexus* L.), Powell amaranth (*Amaranthus powellii* S. Watson), common ragweed (*Ambrosia artemisiifolia* L.) and large crabgrass (*Digitaria sanguinalis* L. Scop.) in 2008. Weed species in 2009 were similar to those in 2008 but also included a large population of wild mustard (*Sinapsis arvensis* L.).

Visual estimates of weed control were made at 7, 14, 21 and 28 days after treatment (DAT) in 2008 and 7, 14 and 21 DAT in 2009 on a scale of 0% (no control) to 100% (complete control) based on injury compared to the untreated control.

### **Greenhouse studies.**

*Plant material.* Common lambsquarters, giant foxtail, and velvetleaf from seed, and Canada thistle (*Cirsium arvense* L. Scop.) from root stock<sup>b</sup> were grown in 9-cm pots containing a commercial potting medium<sup>c</sup> in a greenhouse with temperature maintained at  $23 \pm 3^{\circ}\text{C}$ . Natural light was supplemented by high-pressure sodium lamps producing a photosynthetic photon flux density of  $200 \text{ mol m}^{-2} \text{ s}^{-1}$  with a photoperiod of 16/8 h light/dark. Pots were watered daily to maintain adequate soil conditions for optimum plant growth. Plants were fertilized with 50 ml of fertilizer solution containing 6 mg/L of 20% nitrogen, 20%  $\text{P}_2\text{O}_5$  and 20%  $\text{K}_2\text{O}$  as needed. Weeds were sprayed at 10-12 cm which is considered larger than optimum size which was done to accentuate differences

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between herbicide treatments. Greenhouse grown plants are typically more susceptible to herbicides, however, spraying larger plants can compensate for this. There were four replications per experiment and each experiment was repeated four times. When experiments were conducted on common lambsquarters they were repeated six times because of the susceptibility to pythium (*Pythium spp.*) and fusarium (*Fusarium spp.*) after glyphosate or glufosinate application.

*Herbicide treatments.* Herbicides were applied in a matrix format so a range of possible combinations of each herbicide at each rate were combined. Herbicides were applied as follows; glyphosate<sup>d</sup> at rates of 0, 210, 280, 420, and 840 g ae/ha and glufosinate<sup>e</sup> at rates of 0, 118, 157, 235, and 470 g ai/ha. These rates are representative of 0.0X, 0.25X, 0.33X, 0.5X, and 1X of the label-recommended rate (Anonymous, 2007a; Anonymous, 2007b). Ammonium sulfate was included at 2% v/v in all treatments.

Applications were made using a single-nozzle track sprayer with an 8001 even flat fan nozzle<sup>f</sup> calibrated to deliver 187 L/ha at a pressure of 207 kPa. Visual estimates of weed control were taken at 5, 7, 14, 21 and 28 days after treatment (DAT) on a scale of 0% (no control) to 100% (complete control) based on injury compared with the untreated control. Plant height data was collected and above ground biomass was harvested and immediately weighed at 28 DAT. Plant samples were oven-dried at 50 C for 48 hr and dry weights were recorded.

**Data analysis.** The data analysis was conducted using ANOVA using PROC MIXED in SAS 9.2<sup>9</sup>. Normality of the residuals was evaluated using normal probability and box plots and arc sine data transformations were conducted when there were significant deviations from normality. Homogeneity of variances was evaluated using Levene's test. Herbicide combinations were determined to be antagonistic, synergistic, or additive by comparing the observed plant responses with the expected response when the herbicides are combined. Expected values were calculated using Colby's equation;  $E = X + Y - XY/100$  (Colby, 1967). In the equation, X and Y is the percent growth inhibition by herbicide A and B respectively and E is the expected percent growth inhibition by herbicides A and B combined. Expected and observed responses were compared using Fishers Protected Least Significant Difference (LSD) at  $p = 0.05$  significance. Combinations were determined as antagonistic, synergistic, or additive if the observed response was less than, greater than or similar to the expected response, respectively.

### **Fluorescence Studies.**

**Plant material.** Common lambsquarters (*Chenopodium album* L.) were grown from seed in the greenhouse. Approximately 10 seeds per pot were sown into soil media in 900 mL black plastic pots. Plants were thinned to one per pot upon emergence. Canada thistle (*Cirsium arvense* L. Scop.) were grown from root stock<sup>b</sup> obtained in May of 2008 and transplanted into soil media<sup>c</sup> in 900 mL black plastic pots. These plants were genetically similar. Tillers from stock plants were

transplanted into fresh media and pots. Canada thistle plants were selected for treatment 2 weeks after transplant.

All plants were grown in greenhouses at Michigan State University campus in East Lansing, MI and experiments took place in May of 2009. Natural light was supplemented by high-pressure sodium lamps that produced a photosynthetic photon flux density of  $200 \text{ mol m}^{-2} \text{ s}^{-1}$ . The photoperiod was 16/8 h light/dark, and the temperature was  $23 \pm 3^\circ \text{C}$ . Plants were fertilized with 50 ml of fertilizer solution containing 6 mg/L of 20% nitrogen, 20%  $\text{P}_2\text{O}_5$  and 20%  $\text{K}_2\text{O}$  as needed. Plants were 10-12 cm tall at time of treatment and were randomly assigned to herbicide treatments. Treatments were replicated three times and the experiment repeated three times.

*Herbicide treatments.* Herbicide treatments consisted of glyphosate and glufosinate alone and in combinations at the rates seen in Table 3. Ammonium sulfate (AMS) at 2% v/v was used as an adjuvant. Treatments were applied using a single-tip track sprayer using a TP8001 flat fan nozzle<sup>f</sup> delivering  $187 \text{ L ha}^{-1}$  at a pressure of 207 kPa. Treatments were based on preliminary studies which showed these rates had the highest observable interaction and were also the most economically interesting, such as, a high and a low rate combined, low rates combined and high rates combined.

*Fluorescence measurements.* After herbicide application the plants were immediately returned to the greenhouse and prepared for fluorescence reading. Fluorescence readings were taken at 2, 4, 6, 8, 24, 48 and 72 hours after

treatment (HAT). The second set of fully emerged leaves above the cotyledons, with at least one more set of fully emerged leaves above were selected for fluorescence evaluation. Leafclips<sup>h</sup> were placed in the middle of the selected leaf directly next to the midvein with the least amount of contact with any major veins. The clip has a small shutter plate that must be closed over the leaf once the clip is attached so that light is excluded and dark adaptation begins to take place. The process of dark adaptation varied depending on plant species, ambient light history and whether the plant was stressed. The average time required for dark adaptation in this study was 15 minutes. Once dark adapted, the Handy Pocket PEA<sup>i</sup> optical interface is attached to the clip around the shutter plate, the shutter was opened and high intensity LED light passed through a NIR filter, onto the leaf. Then a highly sensitive PIN photodiode detects the fluorescence signal at 10µs intervals for 1 second. The data obtained in the 1 second period was saved in the Handy Pocket PEA and later downloaded into a computer. Treated plants were visually rated for control, 0 being no control and 100 being complete control, at 7, 14, 21 and 28 days after treatment (DAT). Plant heights were measured at 28 DAT and were then harvested and weighed for fresh weight immediately. Plants harvested were dried at 50°C for 48 hr, dry weights were then determined and samples were discarded.

*Data analysis.* Data were subjected to ANOVA using PROC MIXED in SAS and treatment means for Fv/Fm within species were compared using Fisher's Protected LSD at the  $p = 0.05$  significance level. Data were transformed when necessary for analysis and back-transformed data are presented.

## RESULTS AND DISCUSSION

**Field Studies.** Field studies in 2008 resulted in only one significant interaction; this was due to the high rate of application of herbicides. It was for this reason that a second field study conducted in 2009 which included a greater number of combinations of application rates of glyphosate and glufosinate. Glyphosate combined with glufosinate produced variable results in the field in 2009 which were species dependent. This combination applied to velvetleaf resulted in early synergism, but at 28 DAT all combinations were additive or antagonistic for control (Table 4). Glyphosate and glufosinate combined on common lambsquarters resulted in early synergism and at 28 DAT the high rate of glyphosate with the low rate of glufosinate resulted in antagonism. Where glufosinate was applied at higher rates the antagonism was lost which is consistent with much of the literature (Chuah et al. 2008; Kudsk and Mathiassen 2004; Whitaker 2010). In giant foxtail no early synergism was observed but at 28 DAT antagonism was observed with the application of below labeled rates of glufosinate.

**Greenhouse Studies.** It was hypothesized that combinations including low rates of glufosinate and the range of glyphosate would be antagonistic while combinations with glufosinate at higher rates would be so fast acting that antagonism would no longer be observed when combined with glyphosate. In greenhouse experiments, glyphosate and glufosinate were found to interact antagonistically when applied in combination (Figure 7). This antagonism was



observed at the less than 1X rates of glufosinate in combination with the range of glyphosate rates on Canada thistle (Figure 7a). Results with giant foxtail and common lambsquarters were similar to those of Canada thistle but the combination applied to velvetleaf resulted in antagonism across all rates of glufosinate (Figure 7b). In field experiments results were similar to those found in the greenhouse but with less observable trends due to the complete death of many species attributable to the young growth stage at spraying (Table 4 appendix). There was significant regrowth from combined treatments by velvetleaf, attributed to the failure of glyphosate reaching the actively growing tissue

A second hypothesis was that early synergism between glyphosate and glufosinate would be evident in some species, but by 28 DAT the synergism may no longer be evident. This was observed for the combination of the range of glyphosate rates and the lowest glufosinate rate on giant foxtail. At the 5 DAT observations this combination showed synergism (Figure 8a) which by 28 DAT was no longer evident and in one case replaced with an antagonistic interaction (Figure 8b). Canada thistle, common lambsquarters and velvetleaf had observable early and late antagonism (data not shown). Field studies were not similar to the greenhouse studies. Early synergism was observed in velvetleaf and common lambsquarters but was not seen in giant foxtail (Table 4).

**Fluorescence Studies.** Fv/Fm for Canada thistle was chosen as the parameter to examine for significant differences between herbicide combinations. The

interactions between herbicides are discussed in great detail for both Canada thistle and common lambsquarters in Appendix B. The Fv/Fm of a healthy plant is 0.83, a value smaller than 0.83 is an indication of the inability of the plant, due to injury, to efficiently photosynthesize.

Fv/Fm values following glyphosate and glufosinate combinations applied to Canada thistle were lower than those when glyphosate only and glufosinate only were applied (Figure 9). Differences were dependent upon the rates in the combination and time of measurement (Figure 9). Fv/Fm values after the application of the combination of 1X glyphosate + 0.25X glufosinate were not statistically different from Fv/Fm values following the glufosinate only application at 2 HAT and 72 HAT but were significantly lower than the Fv/Fm values following the glyphosate only application from 4 HAT through 72 HAT (Figure 9a). Fv/Fm values following application of the combination of 0.5X glyphosate + 0.5X glufosinate were significantly lower than the Fv/Fm values after the glufosinate only application at 2 and 8 HAT but were significantly lower than all Fv/Fm values at all HAT after glyphosate only applications (Figure 9b). Fv/Fm values following the application of the combination of 0.25X glyphosate + 1X glufosinate were never significantly different from the Fv/Fm values when glufosinate only was applied (Figure 9c) From 2 to 48 HAT Fv/Fm values of the combined glyphosate and glufosinate were significantly lower than those of the glyphosate only applications (Figure 9c). Fv/Fm values following glyphosate and glufosinate combined treatments were significantly lower than the Fv/Fm values for the control from 4 HAT onward (Figure 10).

Early synergism was observed in the greenhouse for giant foxtail and in the field for common lambsquarters, velvetleaf and Canada thistle. Field studies to determine herbicide interactions generally resulted in more erratic data, possibly due to the effect of the environment on herbicide absorption and translocation. Fluorescence measurements in the greenhouse showed that although glufosinate acted rapidly to break down the PSII system of photosynthesis, it affected herbicide response in combination on these systems. Glufosinate alone and in combination resulted in significantly lower Fv/Fm values than the control or glyphosate alone.

The antagonistic interaction between glyphosate and glufosinate has been observed in other studies on other species and was commonly attributed to the rapid action of glufosinate on the photosynthetic system which reduced glyphosate translocation through the plant (Chuah et al. 2008; Everman et al. 2009; Kudsk and Mathiassen 2004; Whitaker 2010).

The results from these studies show that the combination of glyphosate and glufosinate are antagonistic in common lambsquarters, Canada thistle, giant foxtail and velvetleaf, and that although not indicative of the herbicide interaction, they do show that glufosinate rapidly acts to break down the plant before glyphosate.

## **SOURCE OF MATERIALS**

<sup>a</sup> AirMix 11003, Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434.

<sup>b</sup> Don Penner's Farm Williamston, MI. pennerd@msu.edu.

<sup>c</sup> Baccto® High Porosity Professional Potting Mix, Michigan Peat Co., Houston, TX.

<sup>d</sup> Roundup WeatherMAX®, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167.

<sup>e</sup> Liberty®, Bayer CropScience, Research Triangle Park, NC.

<sup>f</sup> TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

<sup>g</sup> The SAS System for Windows Version 9.2, SAS Institute Inc, 100 SAS Campus Dr., Cary, NC 2751.

<sup>h</sup> Leaf Clips, Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn, Norfolk, U.K.

<sup>i</sup> Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn Norfolk, U.K.

*Table 2. Herbicide combinations applied in field studies 2008 and 2009.<sup>a</sup>*

<b>Herbicides Applied by Year - Field Studies</b>	
<b>2008</b>	<b>2009</b>
Control	Control
0.25X GLY <sup>b</sup>	0.25X GLY
0.33X GLY	0.5X GLY
0.5X GLY	1X GLY
1X GLY	0.25X GLU
1X GLU	0.5X GLU
0.25X GLY + 1X GLU	1X GLU
0.33X GLY + 1X GLU	0.25X GLY + 1X GLU
0.5X GLY + 1X GLU	0.5X GLY + 0.5X GLU
1X GLY + 1X GLU	1X GLY + 0.25X GLU
	1X GLY + 1X GLU

<sup>a</sup> Where 1X = the labeled rate; Glyphosate applied as Roundup WeatherMAX 1X = 840 g ae/ha; Glufosinate applied as LIBERTY 1X = 420 g ai/ha

<sup>b</sup> Abbreviations: GLY, glyphosate; GLU, Glufosinate Thifensulfuron.

**Table 3.** Herbicide combinations applied in fluorescence studies.<sup>a</sup>

<b>Herbicides Applied by Species - Fluorescence Studies</b>	
<b>CIRAR + CHEAL</b>	<b>ABUTH + SETFA</b>
Control	Control
0.25X GLY <sup>b</sup>	0.25X GLY
0.5X GLY	1X GLY
1X GLY	0.25X GLU
0.25X GLU	0.5X GLU
0.5X GLU	1X GLU
1X GLU	0.25X GLU + 1X GLY
0.25X GLU + 1X GLY	
0.5X GLU + 0.5X GLY	
1X GLU + 0.25X GLY	

<sup>a</sup> Where 1X = the labeled rate; glyphosate applied as Roundup WeatherMAX 1X = 840 g ae/ha; Glufosinate applied as LIBERTY 1X = 420 g ai/ha

<sup>b</sup> Abbreviations: GLY, glyphosate; GLU, glufosinate.

**Table 4.** Visual control 7 and 28 days after treatment of velvetleaf, common lambsquarters and giant foxtail when applied with combinations of glyphosate and glufosinate in 2009 field study.<sup>a</sup>

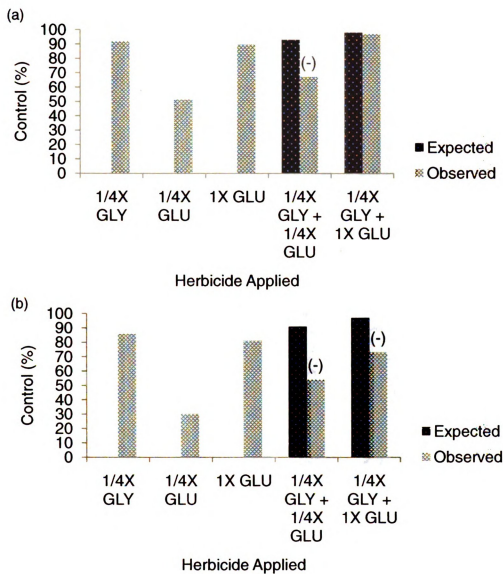
Herbicide Rate <sup>b,c</sup>		Visual Control											
		ABUTH				CHEAL				SETFA			
		7 DAT		28 DAT		7 DAT		28 DAT		7 DAT		28 DAT	
GLY	GLU	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ae/ha	g ai/ha	%											
0	0	-	0	-	0	-	0	-	0	-	0	-	0
210	0	-	38	-	46	-	36	-	48	-	75	-	86
420	0	-	48	-	100	-	29	-	85	-	89	-	99
840	0	-	48	-	100	-	48	-	99	-	80	-	100
0	118	-	30	-	31	-	35	-	31	-	49	-	76
0	235	-	48	-	39	-	46	-	29	-	64	-	73
0	470	-	100	-	70	-	97	-	89	-	94	-	86
210	470	100	99	85	63 (-)	98	95	94	89	98	95	98	95
420	235	73	99 (+)	100	100	62	95 (+)	89	100 (+)	96	91	100	86 (-)
840	118	64	95 (+)	100	90 (-)	66	95 (+)	99	91 (-)	90	95	100	89 (-)
840	470	64	90 (+)	100	100	66	93 (+)	99	100	90	90	100	100
LSD (0.05) <sup>d</sup>		3		2		2		2		2		2	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for a given combination according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: GLY, glyphosate; GLU, glufosinate; ABUTH, velvetleaf; CHEAL, common lambsquarters; SETFA, giant foxtail; DAT, days after treatment; Exp., expected; Obs., observed.

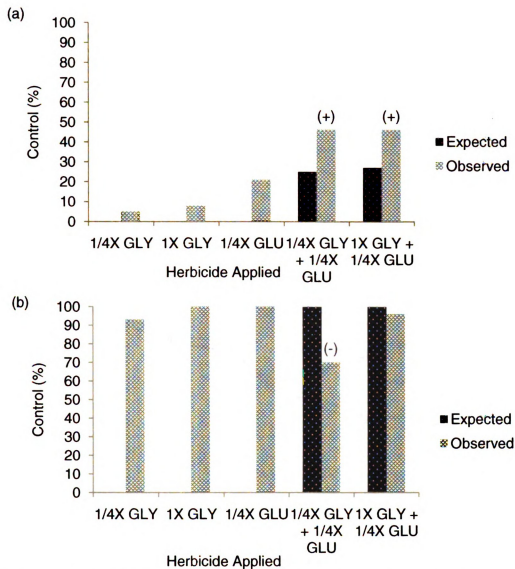
<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2%

<sup>d</sup> LSD values may be used to compare values.

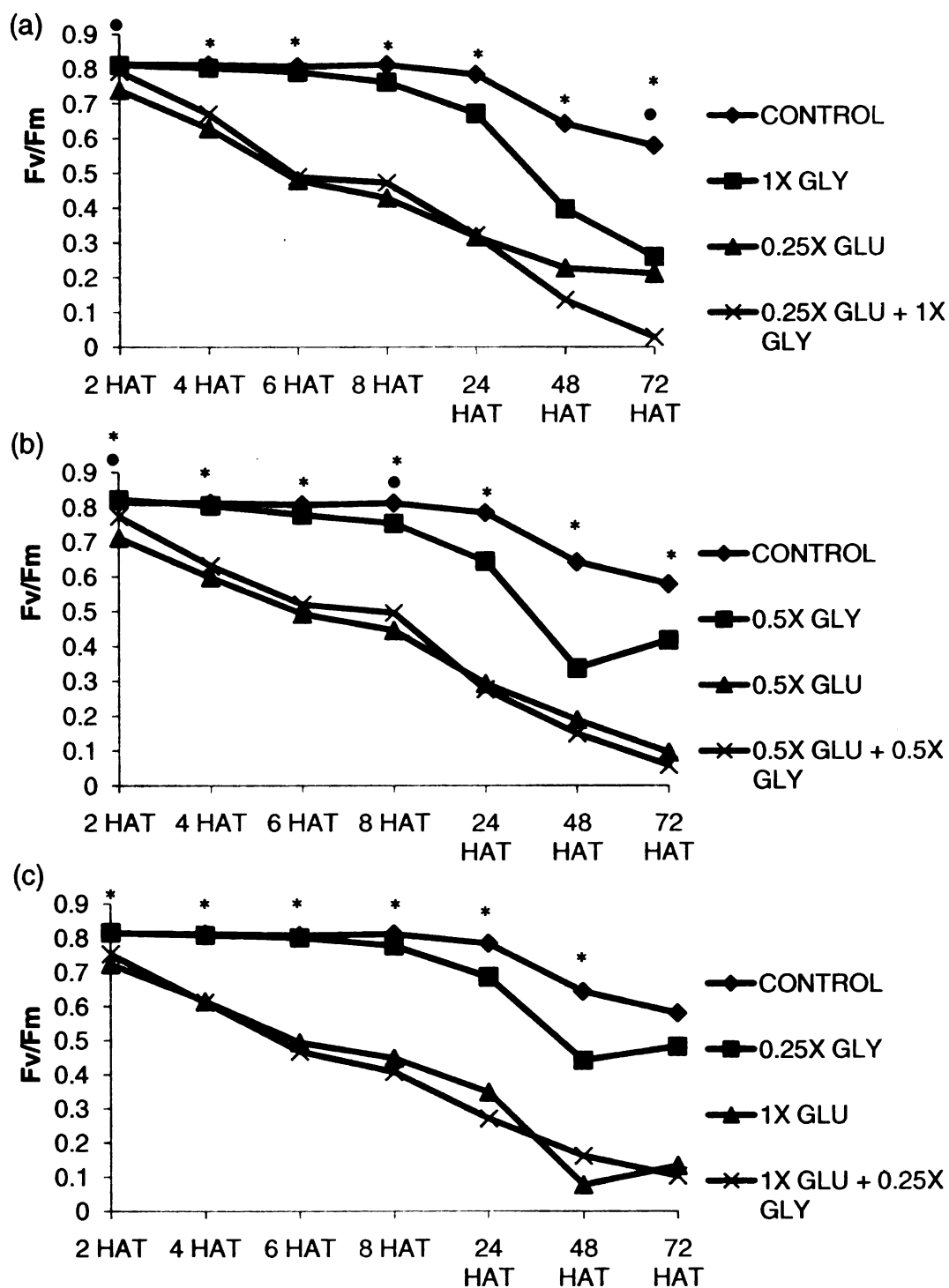


**Figure 7.** Glyphosate(GLY) and glufosinate(GLU) on Canada thistle (a) and velvetleaf (b) visual observations in the greenhouse 28 days after treatment. Antagonism by Colby's method indicated by a (-). LSD = 15 (a) and 10 (b).

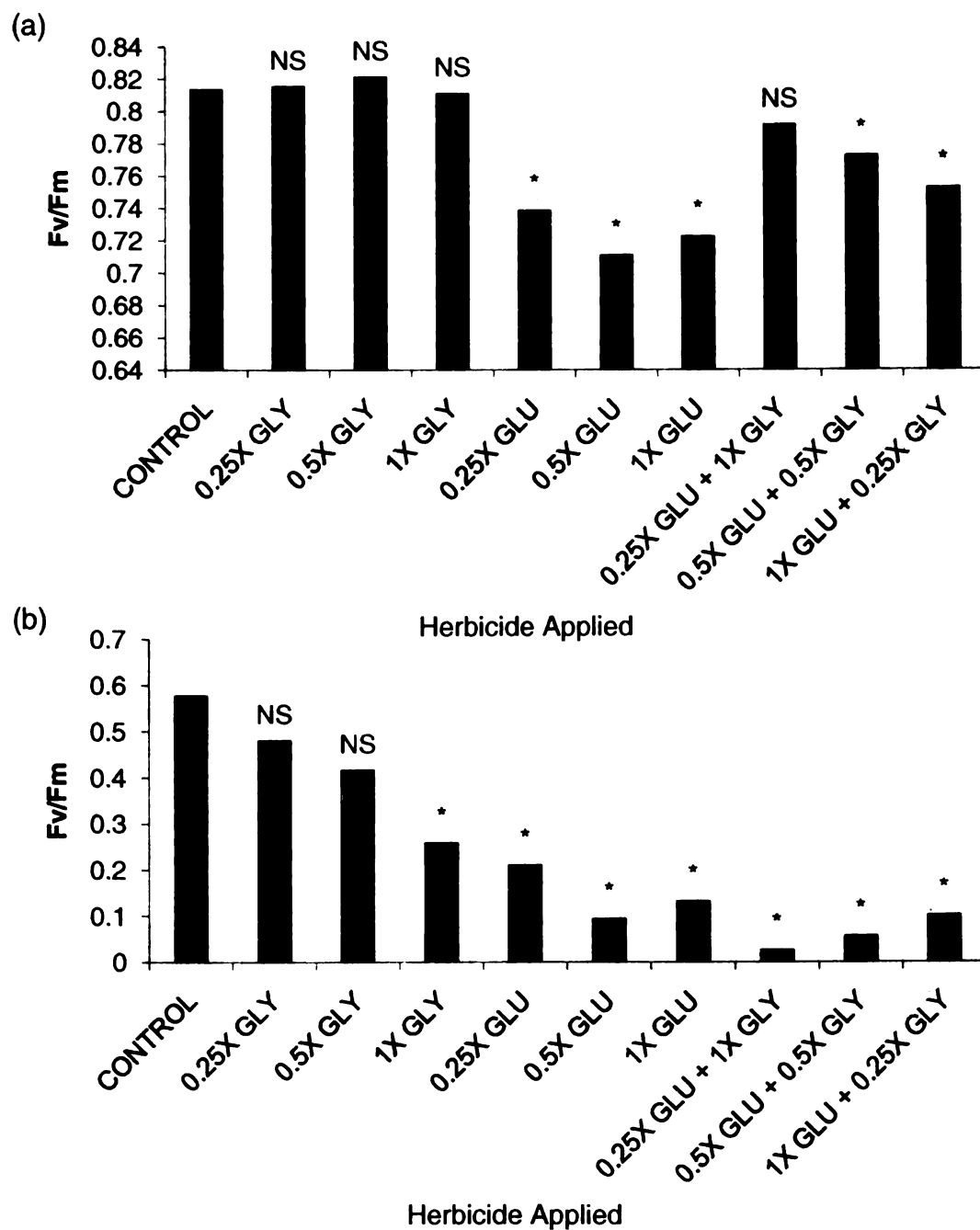




**Figure 8.** Glyphosate(GLY) and glufosinate(GLU) on giant foxtail visual observations in the greenhouse 5 DAT (a) and 28 DAT(b). Antagonism and synergism by Colby's method indicated by a (-) or (+) respectively. LSD = 8.



**Figure 9.** Fv/Fm of glyphosate(GLY) and glufosinate(GLU) on Canada thistle. Where a (\*) indicates the combination is significantly different from glyphosate applied alone and a (•) indicates significantly different from glufosinate applied alone.



**Figure 10.** Fv/Fm of glyphosate and glufosinate on Canada thistle at (a) 2 hours after treatment and (b) 72 hours after treatment. Where a (\*) indicates the combination is significantly different from the control.

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## CHAPTER 4

### EVALUATION OF THE INTERACTION BETWEEN GLYPHOSATE AND THE SULFONYLUREA HERBICIDES CHLORIMURON AND THIFENSULFURON

**Abstract:** The stacking of genes to provide resistance to several herbicides previously injurious to a crop provides new opportunities for control of herbicide resistant weeds. The combination of the non-selective foliar herbicides, glyphosate with the selective sulfonylurea herbicides in a tank mix has the potential to cause unexpected interactions. The objectives of this study were to evaluate the combination of glyphosate and glufosinate on three annual weeds prevalent in Michigan cropping systems; giant foxtail, common lambsquarters, velvetleaf and the perennial weed, Canada thistle and to determine if fluorescence parameters would be indicative of the combined herbicide injury before being visually observable. Field and greenhouse studies showed differential results for the combination of glyphosate and the sulfonylureas on the weed species studied.  $^{14}\text{C}$ -chlorimuron and  $^{14}\text{C}$ -glyphosate were used to determine the basis of a visually observable interaction. The addition of chlorimuron to radiolabeled glyphosate resulted in an increase of glyphosate absorption and translocation in common lambsquarters and giant foxtail and a decrease of translocation in Canada thistle. The addition of glyphosate to radiolabeled chlorimuron caused an increase in chlorimuron absorption in all species while translocation was either similar or lower than when the herbicide was applied alone.



**Nomenclature:** Canada thistle, *Cirsium arvense* L. Scop; common lambsquarters, *Chenopodium album* L.; giant foxtail, *Setaria faberi* Herm.; velvetleaf, *Abutilon theophrasti* Medik; chlorimuron, 2-[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] benzoic acid; glyphosate, N-(phosphonomethyl)-glycine; thifensulfuron, methyl 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate.

**Key words:** Additive effect, antagonism, Colby's, herbicide interaction, reduced rates, synergism, tank-mixing.

The Herbicide Handbook states that chlorimuron may have reduced efficacy when tank-mixing with other herbicides (Sensemen 2007). One study found that chlorimuron and glyphosate were either additive or antagonistic depending on rates applied and species applied to (Starke and Oliver 1998). A recent study found that glyphosate tank-mixed with one-half label rates of chlorimuron gave the highest percent control (80%) of the combinations studied on common lambsquarters (Knezevic et al. 2009). The addition of chlorimuron to glyphosate did not reduce the efficacy of glyphosate in velvetleaf (Jordan et al. 1997). Variation between years was found in a 1996 and 1997 field studies. In 1996 Vidrine et al. 2002 found that at all rates chlorimuron plus glyphosate had greater control than glyphosate applied alone, however this was not observed in 1997 (Vidrine et al. 2002). These studies were not subjected to Colby's analysis of interaction and thus mixed results could have been observed. Tank-mixing glyphosate plus chlorimuron and thifensulfuron resulted in lower soybean yields compared with glyphosate alone but with no significant differences in weed control (Corrigan and Harven 2000).

Tank mixtures of glyphosate and thifensulfuron at a range of rates were additive for control of common lambsquarters (Lich et al. 1997). The basis for the

interaction between these two herbicides was hypothesized to be reduced absorption and translocation of the chlorimuron in combination compared to the chlorimuron applied alone. Absorption of non-formulated glyphosate typically ranged from 25 to 50% while addition of adjuvants in a formulation and ammonium sulfate (AMS) increased absorption up to 90% (Maschhoff et al., 2000; Sprankle et al., 1975; Young et al., 2003). Absorption of glyphosate increased over time with the majority of uptake occurring before 72 hours after treatment, with subsequent translocation through the phloem accumulating in young leaves, roots and meristems (Bromilow et al., 1993). Addition of glyphosate to chlorimuron has been shown to increase the absorption of  $^{14}\text{C}$ -chlorimuron (Starke and Oliver, 1998). Chlorimuron translocation is species dependent and generally low (<50%) (Wilcut et al., 1989).

Glyphosate and chlorimuron, although not primarily Photosystem II (PS II) inhibitors, ultimately cause cellular death resulting in a weakened ability to use or disperse light energy. Changes in fluorescence induction (Kautsky curve) have been used extensively in photosynthesis and herbicide research and are the basis for fluorescence parameters (Abbaspoor and Streibig, 2005; Christensen et al., 2003; Percival and Baker, 1991). The benefits of using fluorescence include its non-invasive procedure, sensitivity to many biotic and abiotic stressors, ease and efficiency and numerous parameters to measure the status of the photosynthetic apparatus (Abbaspoor and Streibig, 2005; Barbagallo et al., 2003; Frankart et al., 2003; Strasser et al., 2000).

Illumination of dark-adapted unstressed leaves produces a rise in chlorophyll fluorescence emission from the ground state ( $F_0$ ) to its maximum value ( $F_m$ ) within one second. An important parameter used in fluorescence research is the  $F_v/F_m$  [ $F_v/F_m = (F_m - F_0)/F_m$ ] parameter (Butler, 1978). The dark adaptation of a leaf allows PSII to be fully reduced at  $Q_A$  and when illuminated the maximum quantum efficiency of the PS II photochemistry can be determined by  $F_v/F_m$ . This parameter is used most often in the literature to indicate plant health with a value of 0.83 indicating no stress to the plant.  $F_v/F_m$  has been used to measure the effect of glyphosate on fluorescence in previous studies. Kirkwood et al 2000 used this parameter and detected treatment differences from the control one day after treatment while neither Olesen and Cedergreen 2010 or Ralph 2000 found any effect of glyphosate on  $F_v/F_m$ .

The objectives of this study were to evaluate potential interactions among four rate matrix combinations of glyphosate with chlorimuron and thifensulfuron in the field and greenhouse, determine if the interactions were significant for antagonism and synergism or just additive, determine if results in the greenhouse were consistent with observations in the field, determine if there were differences between visual control and quantitative measurements, determine if there were different fluorescence responses among species and determine if fluorescence measurements were indicative of early herbicide injury and determine the basis for the interactions observed between glyphosate and chlorimuron with absorption and translocation studies. The following hypothesis were proposed, early synergism between herbicides will be observed in some species , but at 28

DAT synergism may no longer be evident, the interaction of the two different sulfonylureas with the glyphosate may be similar to each other, fluorescence measurements would be indicative of the interaction much earlier than visually observed and absorption and translocation studies would show that reduced absorption and translocation was the basis for the interaction.

## **MATERIALS AND METHODS**

**Field Trial.** Field trials were conducted in 2008 at the Michigan State University Agronomy Research Farm (42°42'42" N, 84°28'13" W) and 2009 at the Michigan State University Plant Pathology Research Center (42°40'59" N, 84°29'5" W) in East Lansing, MI. The soil at the Agronomy Research Farm was a sandy clay loam with 2.6% organic matter and a pH of 6.3. The soil at the Plant Pathology Research Center was a fine sandy loam with a 2 to 6% slope, a pH of 6.9 and 2.5% organic matter. Fields preparation included fall-plowing followed by cultivation in the spring to obtain maximum weed emergence. The experimental design was a randomized complete block in 2008 and 2009. Treatments differed between years and were therefore separated by year and summarized in Table 5.

Herbicide applications were made using a tractor-mounted compressed-air sprayer calibrated to deliver 178 L / ha at 207 kPa through AirMix 11003 nozzles<sup>a</sup>. Common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.) and giant foxtail (*Setaria faberi* Herm.) were the predominant weed species in both years and were the focus in this study. Other weed species present included; redroot pigweed (*Amaranthus retroflexus* L.), Powell amaranth

(*Amaranthus powellii* S. Watson), common ragweed (*Ambrosia artemisiifolia* L.) and large crabgrass (*Digitaria sanguinalis* L. Scop.) in 2008. Weed species in 2009 were similar to those in 2008 but also included a large population of wild mustard (*Sinapsis arvensis* L.).

Visual estimates of weed control were made at 7, 14, 21 and 28 days after treatment (DAT) in 2008 and 7, 14 and 21 DAT in 2009 on a scale of 0% (no control) to 100% (complete control) based on injury compared with the untreated control.

### **Greenhouse studies.**

*Plant Material.* Common lambsquarters, giant foxtail, and velvetleaf from seed, and Canada thistle (*Cirsium arvense* L. Scop.) from root stock<sup>b</sup> were grown in 9-cm pots containing a commercial potting medium<sup>c</sup> in a greenhouse with temperature maintained at  $23 \pm 3^{\circ}\text{C}$ . Natural light was supplemented by high-pressure sodium lamps producing a photosynthetic photon flux density of  $200 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with a photoperiod of 16/8 h light/dark. Pots were watered daily to maintain adequate soil conditions for optimum plant growth. Plants were fertilized with 50 ml of fertilizer solution containing 6 mg/L of 20% nitrogen, 20%  $\text{P}_2\text{O}_5$  and 20%  $\text{K}_2\text{O}$  as needed. Weeds were sprayed at 10-12 cm which is considered larger than optimum size which was done to accentuate differences between herbicide treatments. Greenhouse grown plants are typically more susceptible to herbicides, however, spraying larger plants can compensate for

this. There were four replications per experiment and each experiment was repeated four times. When experiments were conducted on common lambsquarters they were repeated six times because of the susceptibility to pythium (*Pythium spp.*) and fusarium (*Fusarium spp.*) after glyphosate or glufosinate application.

*Herbicide treatments.* Herbicides were applied in a matrix format so all possible combinations of each herbicide at each rate were combined, with the exception of the tank-mixtures of chlorimuron and thifensulfuron, both ALS inhibitors. Herbicides were applied as follows; glyphosate<sup>d</sup> rates of 0, 210, 280, 420, and 840 g ae/ha, chlorimuron-ethyl<sup>e</sup> (hereafter referred to as chlorimuron) rates of 0, 2.2, 2.9, 4.4, and 8.8 g ai/ha and thifensulfuron-methyl<sup>f</sup> (hereafter referred to as thifensulfuron) rates of (0, 1.125, 1.5, 2.25, and 4.5 g ai/ha). These rates are representative of 0.0X, 0.25X, 0.33X, 0.5X, and 1X of the label-recommended rate (Anonymous, 2006a; Anonymous, 2006b; Anonymous, 2007). A crop oil concentrate<sup>g</sup> (COC) was included at 1% v/v in treatments containing only chlorimuron or only thifensulfuron. Ammonium sulfate was included at 2% v/v in all treatments containing glyphosate or glufosinate alone or in combination.

Applications were made using a single-nozzle track sprayer with 8001 even flat fan nozzle<sup>h</sup> calibrated to deliver 187 L/ha at a pressure of 207 kPa. Visual estimates of weed control were taken at 5, 7, 14, 21 and 28 days after treatment

(DAT) on a scale of 0% (no control) to 100% (complete control) based on injury compared to the untreated control. The timing of visual injury ratings was dependent upon the herbicide applied and the weed species. Plant height data were collected and above ground biomass harvested and immediately weighed at 28 DAT. Plant samples were oven-dried at 50°C for 48 hr and dry weights were recorded.

*Data analysis.* The data analysis was conducted using ANOVA in PROC MIXED<sup>i</sup>. Normality of the residuals was evaluated using normal probability and box plots and arc sine data transformations were conducted when there were significant deviations from normality. Homogeneity of variances was evaluated using Levene's test. Herbicide combinations were determined to be antagonistic, synergistic, or additive by comparing the observed plant responses with the expected response when the herbicides are combined. Expected values were calculated using Colby's equation;  $E = X + Y - XY/100$  (Colby, 1967). In the equation, X and Y is the percent growth inhibition by herbicide A and B respectively and E is the expected percent growth inhibition by herbicides A and B combined. Expected and observed responses were compared using Fishers Protected least significant difference (LSD) at  $p = 0.05$  significance. Combinations were determined as antagonistic, synergistic, or additive if the observed response was less than, greater than or similar to the expected response respectively.

### **Fluorescence Studies.**

*Plant material.* Common lambsquarters (*Chenopodium album* L.), were grown from seed in the greenhouse. Approximately 10 seeds each were sown into soil media in 900 mL black plastic pots. Plants were thinned to one per pot upon emergence. Canada thistle (*Cirsium arvense* L. Scop.) were grown from root stock obtained in May of 2008 and transplanted into soil media in 900 mL black plastic pots. These plants were genetically similar. Tillers from stock plants were transplanted into fresh media and pots. Canada thistle plants were selected for treatment 2 weeks after transplant.

All plants were grown in greenhouses at Michigan State University campus in East Lansing, MI and experiments took place in May of 2009. Natural light was supplemented by high-pressure sodium lamps that produced a photosynthetic photon flux density of  $200 \text{ mol m}^{-2} \text{ s}^{-1}$ . The photoperiod was 16/8 h light/dark, and the temperature was  $23 \pm 3^\circ\text{C}$ . Plants were fertilized with 50 ml of fertilizer solution containing 6 mg/L of 20% nitrogen, 20%  $\text{P}_2\text{O}_5$  and 20%  $\text{K}_2\text{O}$  as needed. Plants were 10-12 cm tall at time of treatment and were randomly assigned to herbicide treatments. Treatments were replicated three times and the experiment repeated three times with common lambsquarters and Canada thistle. Treatments were replicated three times and the experiment conducted one time with velvetleaf and giant foxtail.

*Herbicide treatments.* Herbicide treatments consisted of glyphosate and chlorimuron alone and in combinations at the rates seen in Table 6. Ammonium sulfate (AMS) at 2% v/v was used as an adjuvant when glyphosate was applied alone or in combination with chlorimuron. Crop oil concentrate was used at 1%



v/v when chlorimuron was used alone. Treatments were applied using a single-tip track sprayer using a TP8001 flat fan nozzle delivering  $187 \text{ L ha}^{-1}$  at a pressure of 207 kPA. Treatments were based on preliminary studies which showed these rates had the highest observable interaction and were also the most economically interesting, such as, a high and a low rate combined for each herbicide respectively, low rates combined and high rates combined.

*Fluorescence measurements.* After herbicide application the plants were immediately returned to the greenhouse and prepared for fluorescence reading. Fluorescence readings were taken at 2, 4, 6, 8, 24, 48 and 72 hours after treatment (HAT). The second set of fully emerged leaves above the cotyledons, with at least one more set of fully emerged leaves above were selected for fluorescence evaluation. Leafclips<sup>j</sup> were placed in the middle of the selected leaf directly next to the midvein with the least amount of contact with any major veins. The clip has a small shutter plate must should be closed over the leaf once the clip is attached so that light is excluded and dark adaptation begins to take place. The process of dark adaptation varied depending on plant species, ambient light history and whether the plant was stressed. The average time required for dark adaptation in this study was 15 minutes. Once dark adapted, the Handy Pocket PEA<sup>k</sup> optical interface is attached to the clip around the shutter plate, the shutter was opened and high intensity LED light passed through a NIR filter, onto the leaf. Then a highly sensitive PIN photodiode detects the fluorescence signal at  $10\mu\text{s}$  intervals for 1 second. The data obtained in the 1 second period was saved in the Handy Pocket PEA and later downloaded into a computer. Treated plants

were visually rated for control, 0 being no control and 100 being complete control, at 7, 14, 21 and 28 days after treatment (DAT). Plant heights were measured at 28 DAT and were then harvested and weighed for fresh weight immediately. Plants harvested were dried at 50°C for 48 hr, dry weights were then determined and samples were discarded.

*Data analysis.* Data were subjected to ANOVA using PROC MIXED in SAS and treatment means for Fv/Fm within species were compared using Fisher's Protected LSD at the  $p = 0.05$  significance level. Data were transformed when necessary for analysis and back-transformed data are presented.

### **Absorption and Translocation of Glyphosate and Chlorimuron**

*Plant Material.* Common lambsquarters (*Chenopodium album* L.) and giant foxtail (*Setaria faberi* Herm.) from seed, and Canada thistle (*Cirsium arvense* L. Scop.) from root stock were grown in 9-cm pots containing a commercial potting medium in a greenhouse with temperature maintained at  $23 \pm 3^\circ\text{C}$ . Natural light was supplemented by high-pressure sodium lamps producing a photosynthetic photon flux density of  $200 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with a photoperiod of 16/8 h light/dark. Weeds were sprayed at 10-12 cm which is considered larger than optimum size which was done to accentuate differences between herbicide treatments. Plants were randomly assigned to herbicide treatments and harvest times. Experiments were replicated four times and conducted twice.

*Uptake and translocation.* The first fully expanded leaf on each plant of each species was chosen for  $^{14}\text{C}$  treatment. This leaf was marked and carefully wrapped with aluminum foil before broadcast herbicide application. Herbicide combinations of glyphosate and chlorimuron were applied to plants at the 6 to 8 leaf stage using a single-tip track sprayer with a 8001E flat fan nozzle delivering  $187 \text{ L ha}^{-1}$  at a pressure of 207 kPA. After application of the herbicides, plants were moved to the greenhouse and the aluminum foil removed. The  $^{14}\text{C}$  spotting solution contained  $^{14}\text{C}$ -glyphosate with a specific activity of  $5 \text{ kBq mg}^{-1}$  or  $^{14}\text{C}$ -chlorimuron with a specific activity of  $2.4 \text{ kBq mg}^{-1}$  and herbicide solution to bring the spotting solution to the combined rates (Table 7). Ten  $1\mu\text{L}$  drops were then applied to the adaxial leaf surface of the second fully expanded leaf, five on either side of the leaf midrib, to deliver a total of  $10\mu\text{L}$  of  $^{14}\text{C}$ -labeled and broadcast spray mix. Following treatment the treated leaves were washed off and plants were harvested at 6, 24 and 48 hours after treatment (HAT). At each of the harvest times treated leaves were removed and rinsed with a 1:1 methanol : distilled water mix with 0.25% v/v non-ionic surfactant (NIS), to remove any unabsorbed herbicide. The rinsate was radioassayed by liquid scintillation spectrophotometry (LSS). The quantity of  $^{14}\text{C}$ -glyphosate in the rinsate was compared to the amount of  $^{14}\text{C}$ -glyphosate applied for each treatment. At the time of rinsing, plants are separated into treated leaf (TL), above treated leaf (ATL), and below treated leaf (BTL). ATL and BTL were separated at the leaf

axial of the TL. The portion of  $^{14}\text{C}$ -glyphosate translocated was calculated by subtracting the amount in the treated leaf from the amount absorbed. Plants were then put into the dryer at 50°C for 48 hours, ground using a mortar and pestle and 1g of material per sample weighed and oxidized.

*Data analysis.* Data were analyzed using ANOVA in PROC MIXED in SAS

9.2. Normality of the residuals was evaluated using normal probability and box plots. Homogeneity of variances was evaluated using Levene's test. No significant differences were found between the duplicate experiments. Mean separation was achieved with Fisher's Protected LSD Test at the  $p = 0.05$  significance level.

## RESULTS AND DISCUSSION

**Field Studies.** Field studies in 2008 resulted in only one significant interaction; due to the high rate of application of herbicides. Thus a second field study was conducted in 2009 with a greater number combination of application rates of glyphosate and chlorimuron. Early synergism and antagonism were observed in all species at all the rates tested. For all species studied the 7 DAT observations resulted in more statistically significant interactions than the 28 DAT observations. Giant foxtail was the only species to have observable interactions at the 7 DAT observation and no significant interactions at the 28 DAT observations (Table 8).

**Greenhouse Studies.**

The sulfonylurea herbicides combined with glyphosate applied to common lambsquarters resulted in similar interactions as hypothesized. At 28 DAT both of these combinations showed similar synergistic combinations (Figure 11). However the combination with thifensulfuron showed more synergism at all times and also had antagonism with two combinations at 28 DAT (data not shown). Fresh weight reduction and height reduction data indicated greater weed control was achieved with the thifensulfuron than chlorimuron combined with glyphosate, however, the visual control observations showed exactly the opposite (data not shown). Chlorimuron treated plants showed greater leaf desiccation; however plant weight and height of plants receiving chlorimuron plus glyphosate were greater than when thifensulfuron was applied with glyphosate (Figure 11). Although there were a few examples of when the sulfonylurea herbicides acted similarly in combination with glyphosate, there were more instances where they were dissimilar for weed control. The combination of the sulfonylurea herbicides and glyphosate resulted in more significant interactions in the chlorimuron combination than the thifensulfuron combination on Canada thistle, velvetleaf and giant foxtail (data not shown).

The third hypothesis was that the combination of glyphosate and chlorimuron interactions would be species dependent. Antagonism was apparent from the height reduction data for Canada thistle and velvetleaf and the visual control data for giant foxtail (Figure 12). Visual control data from all other species showed species dependent antagonism and synergism less than the quantitative observations (data not shown). The combined application of glyphosate and

chlorimuron to common lambsquarters showed significant synergism in data collected at 28 DAT (Figure 12). Based on these results the combination of glyphosate and chlorimuron was chosen for absorption and translocation studies.

**Fluorescence Studies.** Fv/Fm for Canada thistle was chosen as the parameter to examine for significant differences between herbicide combinations. The interactions between herbicides are discussed in great detail for both Canada thistle and common lambsquarters in Appendix B. The Fv/Fm of a healthy plant is 0.83, a value smaller than 0.83 is an indication of the inability of the plant, due to injury, to efficiently photosynthesize.

Fv/Fm for plants receiving glyphosate and chlorimuron combinations were no different than for glyphosate alone at all HAT (data not shown). Fv/Fm values after combined treatments were no different from those of the control treatments at 2 HAT. However, at 72 HAT many Fv/Fm values after application of glyphosate and chlorimuron combinations were significantly lower than the Fv/Fm of the control and chlorimuron only treatments (Figure 13). This indicates that although glyphosate alone reduces the photosynthetic ability of the plant (as seen by a reduction in the Fv/Fm with glyphosate alone and in combination) the addition of chlorimuron does not significantly change the results. Glyphosate alone is responsible for the reduced photosynthetic ability of the plant within the period of our study (72 HAT).

### **Absorption and Translocation**

*<sup>14</sup>C-glyphosate.* Absorption of <sup>14</sup>C-glyphosate ranged from 53 to 75% of the applied radioactive material increasing from 6 HAT to 48 HAT for the majority of rates and combinations in all species (Figure 14). The addition of chlorimuron caused a significant increase in absorption of the <sup>14</sup>C-glyphosate in common lambsquarters and giant foxtail (Figure 14b,c). The absorption increased with the combination evident in common lambsquarters may explain the observed synergism in weed control but the increase in absorption evident in giant foxtail does not explain the observed antagonism (Figure 14).

Translocation of <sup>14</sup>C-glyphosate out of the treated leaf ranged from 50 to 75% of the absorbed radioactive material increasing from 6 HAT to 48 HAT for the majority of rates and combinations in all species (Figure 15). The addition of chlorimuron increased the translocation of <sup>14</sup>C-glyphosate in common lambsquarters and giant foxtail while reduced translocation was seen with one combination in Canada thistle (Figure 15). The reduced translocation of <sup>14</sup>C-glyphosates in Canada thistle is consistent with the observed antagonism between glyphosate and chlorimuron (data not shown).

*<sup>14</sup>C-Chlorimuron.* Absorption of <sup>14</sup>C-chlorimuron ranged from 85 to 99% of the applied increasing from 6 HAT to 48 HAT for the majority of rates and combinations in all species (Figure 16). The addition of glyphosate to <sup>14</sup>C-chlorimuron increased absorption in all species at all HAT (Figure 16).

Translocation of  $^{14}\text{C}$ -chlorimuron out of the treated leaf ranged from 20 to 75% of the absorbed (Figure 17). Translocation was greatest in common lambsquarters and lowest in giant foxtail (Figure 17b,c). Translocation of  $^{14}\text{C}$ -chlorimuron did not increase from 6 HAT to 48 HAT but appeared evident (Figure 17b). The addition of glyphosate to  $^{14}\text{C}$ -chlorimuron reduced translocation in all species, and was dependent upon time harvested (Figure 17).

*Comparison with visual observations.* Antagonism was observed between chlorimuron and glyphosate on Canada thistle in previous greenhouse studies (data not shown). Absorption and translocation of glyphosate increased with the addition of chlorimuron, not reflective of the observed antagonism (Figure 14a, 15a). Although absorption of chlorimuron increased with the addition of glyphosate, reduced translocation of chlorimuron out of the treated leaf was observed when combined with glyphosate in Canada thistle and could be the cause of the observed antagonism (Figure 17a). Synergism between low rates of glyphosate and the range of chlorimuron rates was observed in previous greenhouse studies on common lambsquarters (Chapter 2, Figure 3). Absorption and translocation of both  $^{14}\text{C}$ -glyphosate and radio labeled  $^{14}\text{C}$ -chlorimuron increased with the addition of chlorimuron and glyphosate, respectively, in common lambsquarters (Figure 14b, 15b, 16b, 17b). The increase in absorption and translocation was across all rates of glyphosate although synergism was only observed at low rates of glyphosate. The synergism between chlorimuron and glyphosate on common lambsquarters may be due to the increased



absorption and translocation of both herbicides in combination. Antagonism was observed between glyphosate and chlorimuron in previous greenhouse studies on giant foxtail (Table 9). Absorption and translocation of glyphosate combined with chlorimuron was greater than glyphosate applied alone in giant foxtail (Figure 14c, 15c). Although absorption of chlorimuron was greater when glyphosate was added, translocation out of the treated leaf did not increase, possibly the basis of the observed antagonism in weed control (Figure 16c, 17c).

In the greenhouse studies, the two sulfonylurea herbicides responded similarly in combinations with glyphosate but this was species dependent. Synergism was observed in combinations with glyphosate; however, the thifensulfuron combination provided greater weed control than the chlorimuron. In field studies early synergism or antagonism was observed in all species except giant foxtail. Results for some combinations were similar in field and greenhouse studies but due to the differential environment in the field, correlation between the two was not possible. Fluorescence measurements of glyphosate and chlorimuron combined did not indicate an interaction prior to 72 HAT; however glyphosate reduced the fluorescence beginning at 24 HAT. Fluorescence of plants treated with chlorimuron indicated no injury to the photosynthetic system prior to 72 HAT.

Absorption and translocation data for both  $^{14}\text{C}$ -glyphosate and  $^{14}\text{C}$ -chlorimuron showed that increased absorption was evident with the addition of the other herbicide. The increase in absorption could have been due to the addition of AMS when glyphosate was paired with  $^{14}\text{C}$ -chlorimuron and the

formulation effect of chlorimuron when paired with  $^{14}\text{C}$ -glyphosate. Interactions between glyphosate and chlorimuron were apparent in absorption and translocation data for some species but the basis for all the visually observed symptoms of interactions was not fully explained by this study. Additional studies should be completed to further look at the effect of adjuvant additions and formulations on these species.

The interaction of glyphosate and the sulfonylurea herbicides although not always, generally resulted in antagonistic interactions in the species studied. Fluorescence measurements did not indicate an early interaction but were indicative of glyphosate injury. Absorption and translocation studies with both  $^{14}\text{C}$ -glyphosate and  $^{14}\text{C}$ -chlorimuron applied alone and in combination were insufficient to explain the basis of the observed interaction.

## **SOURCES OF MATERIALS**

<sup>a</sup> AirMix 11003, Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434.

<sup>b</sup> Don Penner's Farm Williamston, MI. pennerd@msu.edu.

<sup>c</sup> Baccto® High Porosity Professional Potting Mix, Michigan Peat Co., Houston, TX.

<sup>d</sup> Roundup WeatherMAX®, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167.

<sup>e</sup> CLASSIC®, DuPont Agricultural Products, Newark, DE.

<sup>f</sup> HARMONY®, DuPont Agricultural Products, Newark, DE.

<sup>g</sup> Crop oil concentrate. Loveland Products, Inc. PO Box 1286 Greely, CO 80632.

<sup>h</sup> TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

<sup>i</sup> The SAS System for Windows Version 9.2, SAS Institute Inc, 100 SAS Campus Dr., Cary, NC 2751.

<sup>j</sup> Leaf Clips, Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn, Norfolk, U.K.

<sup>k</sup> Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn Norfolk, U.K.

*Table 5. Herbicide combinations applied in field studies 2008 and 2009.<sup>a</sup>*

<b>Herbicides Applied by Year - Field Studies</b>	
<b>2008</b>	<b>2009</b>
Control	Control
0.25X GLY <sup>b</sup>	0.25X GLY
0.33X GLY	0.5X GLY
0.5X GLY	1X GLY
1X GLY	0.25X CHL
1X CHL	0.5X CHL
0.25X GLY + 1X CHL	1X CHL
0.33X GLY + 1X CHL	0.25X GLY + 0.25X CHL
0.5X GLY + 1X CHL	0.25X GLY + 0.5X CHL
1X GLY + 1X CHL	0.25X GLY + 1X CHL
1X THI	0.5X GLY + 0.25X CHL
1X THI + 0.25X GLY	0.5X GLY + 0.5X CHL
1X THI + 0.33X GLY	0.5X GLY + 1X CHL
1X THI + 0.5X GLY	1X GLY + 0.5X CHL
1X THI + 1X GLY	1X GLY + 1X CHL

<sup>a</sup> Where 1X = the labeled rate; Glyphosate applied as Roundup WeatherMAX 1X = 840 g ae/ha; Chlorimuron applied as CLASSIC 1X = 8.8 g ai/ha; Thifensulfuron applied as HARMONY DS 1X = 4.5 g ai/ha

<sup>b</sup> Abbreviations: GLY, glyphosate; CHL, Chlorimuron; THI, Thifensulfuron.

**Table 6.** Herbicide combinations applied in fluorescence studies.<sup>a</sup>

<b>Herbicides Applied by Species - Fluorescence Studies</b>	
<b>CIRAR + CHEAL</b>	<b>ABUTH + SETFA</b>
Control	Control
0.25X GLY <sup>b</sup>	0.25X GLY
0.5X GLY	1X GLY
1X GLY	1X CHL
0.25X CHL	0.25X GLY + 1X CHL
0.5X CHL	
1X CHL	
0.25X GLY + 0.25X CHL	
0.25X GLY + 1X CHL	
0.5X GLY + 0.5X CHL	
1X GLY + 0.25X CHL	
1X GLY + 0.5X CHL	
1X GLY + 1X CHL	

<sup>a</sup> Where 1X = the labeled rate; glyphosate applied as Roundup WeatherMAX 1X = 840 g ae/ha; chlorimuron applied as CLASSIC 1X = 8.8 g ai/ha.

<sup>b</sup> Abbreviations: GLY, glyphosate; CHL, chlorimuron.

*Table 7.* Herbicide treatments for  $^{14}\text{C}$  absorption and translocation studies.

$^{14}\text{C}$ -Chlorimuron Applied	$^{14}\text{C}$ -Glyphosate Applied
0.25X Chlorimuron	0.25X Glyphosate
0.5X Chlorimuron	0.5X Glyphosate
1X Chlorimuron	1X Glyphosate
0.25X Glyphosate + 1X Chlorimuron	0.25X Glyphosate + 1X Chlorimuron
1X Glyphosate + 0.25X Chlorimuron	1X Glyphosate + 0.25X Chlorimuron
0.5X Glyphosate + 0.5X Chlorimuron	0.5X Glyphosate + 0.5X Chlorimuron
1X Glyphosate + 1X Chlorimuron	1X Glyphosate + 1X Chlorimuron
For Chlorimuron X= 8.8 g ai/ha	
For Glyphosate X= 840 g ae/ha	

**Table 8.** Visual control 7 and 28 days after treatment of velvetleaf, common lambsquarters and giant foxtail when applied with combinations of glyphosate and chlorimuron in 2009 field study.<sup>a</sup>

Herbicide Rate <sup>b,c,d</sup>		Visual Control											
		ABUTH				CHEAL				SETFA			
		7 DAT		28 DAT		7 DAT		28 DAT		7 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
GLY	CHL	%											
g ae/ha													
0	0	-	0	-	0	-	0	-	0	-	0	-	0
210	0	-	38	-	46	-	36	-	48	-	75	-	86
420	0	-	48	-	100	-	29	-	85	-	89	-	99
840	0	-	48	-	100	-	48	-	99	-	80	-	100
0	2.2	-	43	-	29	-	16	-	30	-	6	-	35
0	4.4	-	26	-	39	-	15	-	44	-	2	-	51
0	8.8	-	39	-	38	-	23	-	25	-	25	-	46
210	2.2	64	42 (-)	62	78 (+)	47	36 (-)	63	71 (+)	77	91 (+)	91	94
210	4.4	54	26 (-)	67	59 (-)	46	37 (-)	71	72	76	86 (+)	94	94
210	8.8	61	81 (+)	68	98 (+)	51	55	61	95 (+)	81	71 (+)	93	94
420	2.2	70	92 (+)	100	90 (-)	40	83 (+)	89	86	90	90	99	100
420	4.4	61	29 (-)	100	95	39	91 (+)	92	81 (-)	89	90	99	98
420	8.8	67	38 (-)	100	100	45	57 (+)	89	72 (+)	92	86	99	100
840	4.4	61	61	100	99	55	36 (-)	99	83 (-)	81	89 (-)	100	100
840	8.8	67	92 (+)	100	100	59	60	99	98	85	91	100	100
LSD (0.05) <sup>e</sup>		3		2		2		2		2		2	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for a given combination according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: GLY, glyphosate; CHL, chlorimuron; ABUTH, velvetleaf; CHEAL, common lambsquarters; SETFA, giant foxtail; DAT, days after treatment; Exp., expected; Obs.,

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2%

<sup>d</sup> Treatments containing only chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 9** Percent control compared to the untreated for combinations of glyphosate and chlorimuron on giant foxtail 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Giant foxtail							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Chlorimuron	Glyphosate	%							
g ai/ha	g ae/ha								
0	0	-	0	-	0	-	0	-	0
2.2	0	-	0	-	0	-	4	-	15
2.93	0	-	7	-	4	-	0	-	21
4.4	0	-	7	-	4	-	3	-	20
8.8	0	-	7	-	8	-	6	-	29
0	210	-	16	-	66	-	71	-	40
0	280	-	19	-	80	-	88	-	50
0	420	-	18	-	93	-	87	-	68
0	840	-	41	-	98	-	85	-	91
2.2	210	16	18	66	69	72	84 (+)	49	44
2.93	210	21	20	68	88 (+)	71	89 (+)	53	45
4.4	210	21	9 (-)	68	89 (+)	72	85 (+)	52	45
8.8	210	21	10 (-)	69	59 (-)	73	86 (+)	57	51
2.2	280	19	13 (-)	80	86	89	89	57	55
2.93	280	24	9 (-)	81	91 (+)	88	93	61	55
4.4	280	24	13 (-)	81	89	88	91	60	55
8.8	280	24	11 (-)	81	92 (+)	89	92	64	55
2.2	420	18	11 (-)	93	97	87	91	72	70
2.93	420	24	23	93	95	87	89	75	69
4.4	420	24	18 (-)	93	98	87	88	75	77
8.8	420	24	19 (-)	94	96	88	89	77	72
2.2	840	41	29 (-)	98	98	86	89	92	89
2.93	840	45	33 (-)	98	98	85	87	93	93
4.4	840	45	31 (-)	98	99	85	91	93	86
8.8	840	45	41	98	100	86	91	93	93
LSD (0.05) <sup>e</sup>		5		9		9		4	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

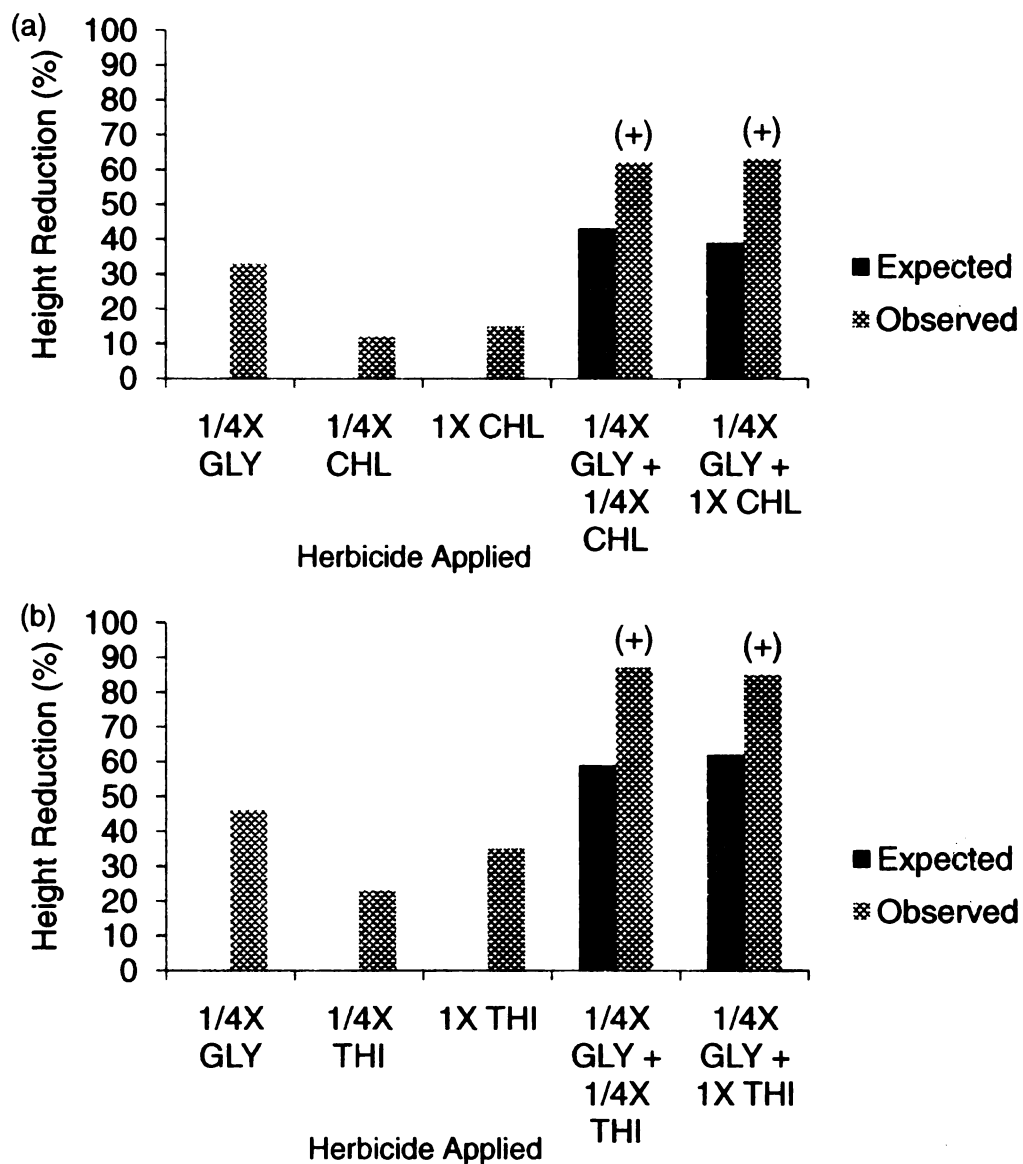
<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.





**Figure 11.** Chlorimuron(CHL) and glyphosate(GLY) (a) and thifensulfuron(THI) and glyphosate (b) on common lambsquarters % height reduction from the control in the greenhouse 28 days after treatment. Synergism by Colby's method indicated by a (+). LSD = 5 for 7 DAT and LSD = 8 for 28 DAT.

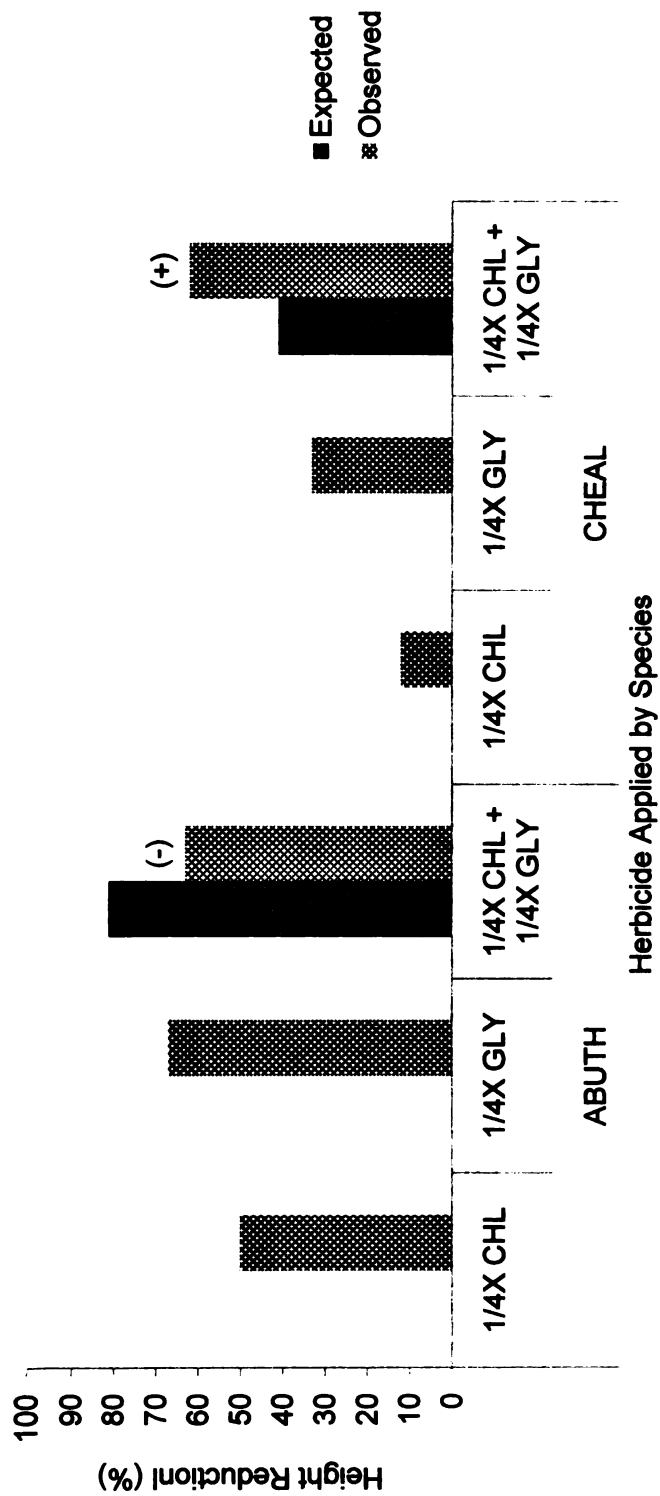


Figure 12. Chlorimuron(CHL) and glyphosate(GLY) on velvetleaf(ABUTH) and common lambsquarters(CHEAL) % height reduction in the greenhouse 28 days after treatment. Antagonism and synergism by Colby's method indicated by a (-) or (+) respectively. LSD = 9 for ABUTH and LSD = 17 for CHEAL.

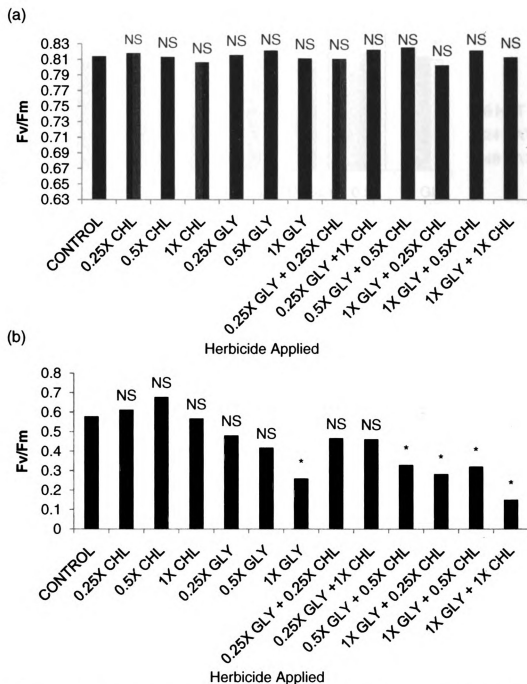


Figure 13. Fv/Fm of glyphosate (GLY) and chlorimuron (CHL) on Canada thistle at (a) 2 hours after treatment and (b) 72 hours after treatment. Where a \* indicates significant difference from the control. (a) LSD = 0.0356 and (b) LSD = 0.1795.

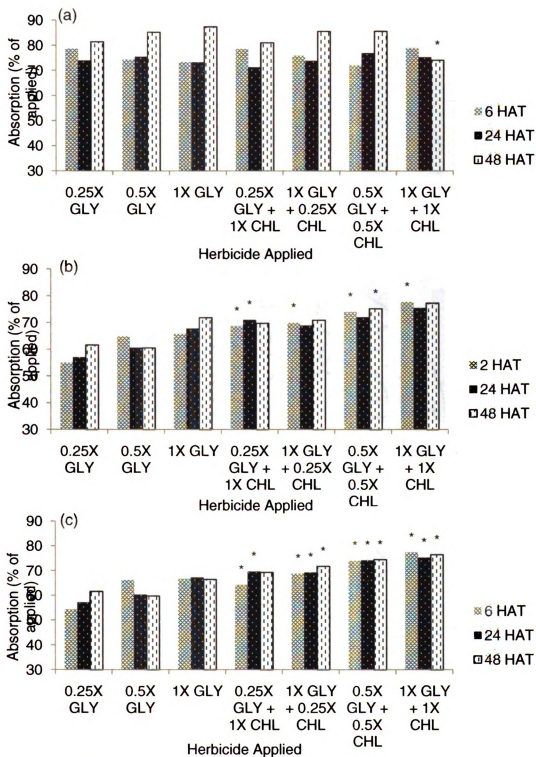


Figure 14.  $^{14}\text{C}$ -glyphosate absorption in (a) Canada thistle, (b) common lambsquarters and (c) giant foxtail. A (\*) indicates significantly different from herbicide alone at that time. Abbreviations: CHL, chlorimuron; DAT, days after treatment; GLY, glyphosate.

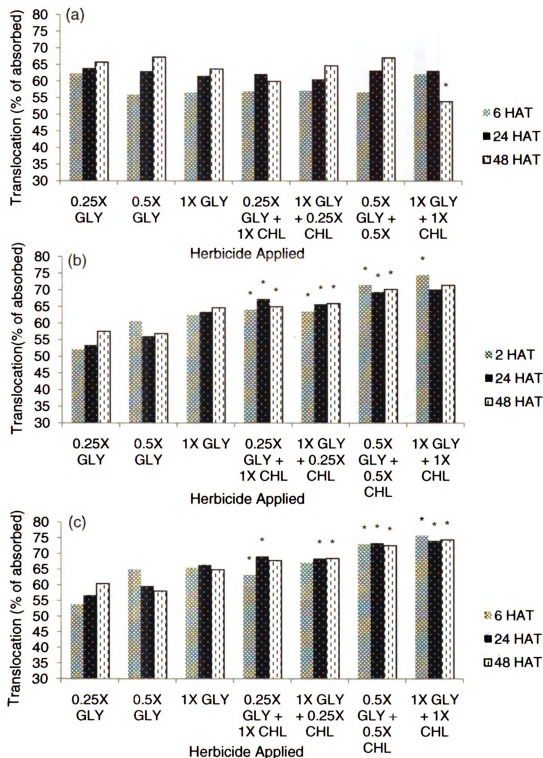
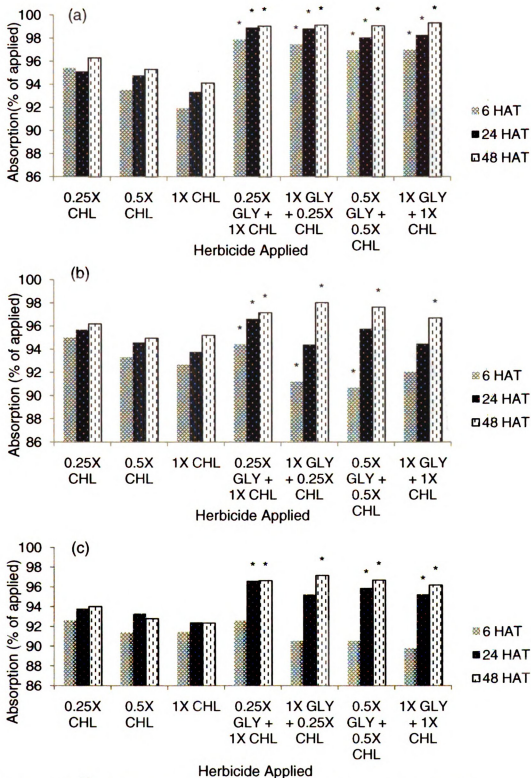


Figure 15.  $^{14}\text{C}$ -glyphosate translocation out of the treated leaf by (a) Canada thistle, (b) common lambsquarters and (c) giant foxtail. A (\*) indicates significantly different from herbicide alone at that time. Abbreviations: CHL, chlorimuron; DAT, days after treatment; GLY, glyphosate.



**Figure 16.** <sup>14</sup>C-chlorimuron absorption in (a) Canada thistle, (b) common lambsquarters and (c) giant foxtail. A (\*) indicates significantly different from herbicide alone at that time. Abbreviations: CHL, chlorimuron; DAT, days after treatment; GLY, glyphosate.

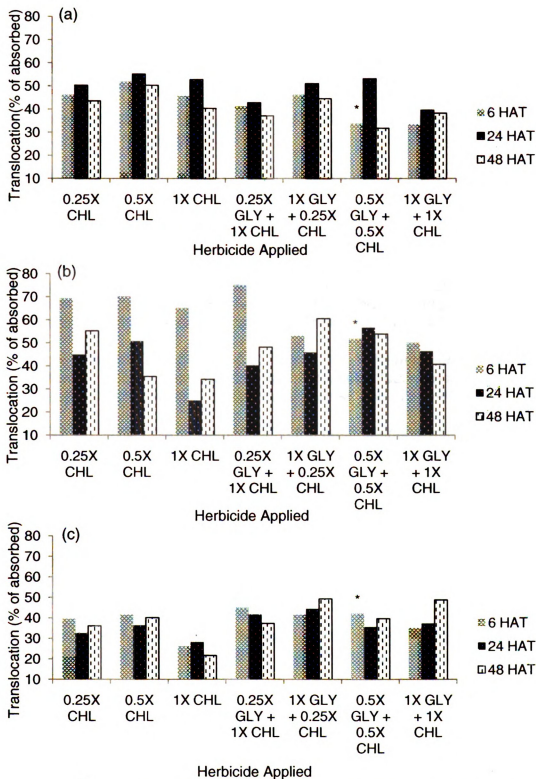


Figure 17. <sup>14</sup>C-chlorimuron translocation out of the treated leaf by (a) Canada thistle, (b) common lambsquarters and (c) giant foxtail. A (\*) indicates significantly different from herbicide alone at that time. Abbreviations: CHL, chlorimuron; DAT, days after treatment; GLY, glyphosate.

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## CHAPTER 5

### EVALUATION OF THE COMBINATION OF GLUFOSINATE AND THE SULFONYLUREA HERBICIDES CHLORIMURON AND THIFENSULFURON.

**Abstract:** The stacking of genes to provide resistance to several herbicides previously injurious to a crop provides new opportunities for control of a larger range of species and also herbicide resistant weeds. Specifically the opportunity may exist to control sulfonylurea resistant weeds with glufosinate. The combination of these herbicides in a tank mix also has the potential to cause unexpected interactions. Studies were conducted in the field in 2008 and 2009 and in the greenhouse from 2008 to 2010 to evaluate the combination of glufosinate and the sulfonylurea herbicides chlorimuron and thifensulfuron on three annual weeds prevalent in Michigan cropping systems; giant foxtail, common lambsquarters, velvetleaf, and the perennial weed, Canada thistle. In the greenhouse, the interaction of glufosinate with the sulfonylurea herbicide resulted in early synergism, late antagonism and late synergism. These results were dependent upon the combination applied and the species. Similarities were found between the two sulfonylurea herbicides in combination with glufosinate and similarities were also found between field and greenhouse results.

**Nomenclature:** Canada thistle, *Cirsium arvense* L. Scop; common lambsquarters, *Chenopodium album* L.; giant foxtail, *Setaria faberi* Herm.; velvetleaf, *Abutilon theophrasti* Medik; chlorimuron, 2-[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] benzoic acid; glufosinate, 2-amino-4-(hydroxy-methyl-phosphoryl)butanoic acid; thifensulfuron, methyl 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate.

**Key words:** Additive effect, antagonism, Colby's, herbicide interaction, reduced rates, synergism, tank-mixing.

Tank-mixing of herbicides may provide producers the opportunity to increase the range of species controlled by combining multiple herbicides with different modes of action in the tank-mix. The interaction between glufosinate and chlorimuron has been extensively studied with differential results depending on the herbicide rate, stage of weed, method of application and species (Starke and Oliver, 1998; Vidrine et al., 2002).

Glufosinate combined with chlorimuron and metribuzin resulted in an additive effect in sicklepod (*Senna obtusifolia* L. H.S. Iriwn & Barneby ), antagonism at low glufosinate rates in entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula*) and antagonism at high glufosinate rates in johnsongrass (*Sorghum halepense* L. Pers. ) (Hydrick and Shaw 1994, 1995). The addition of a selective foliar herbicide to the non-selective glufosinate often resulted in antagonism but could be overcome by a high application rate of glufosinate especially for the broadleaf weeds studied. No known studies examined the interaction between glufosinate and thifensulfuron.

Desired weed control with a combination of herbicides with one application can be cost effective. Reduced rates can lead to reduced efficacy and can occur in the field for a number of reasons. Accidental mixing, misapplication, a rain event during the rain free period and weather conditions can prevent a producer from getting into the field at an optimal weed size. Also, since weeds do not emerge at the same time, there are usually weeds at variable stages in the field

at time of postemergence application. Reduced rates may result in a reduction of crop yield due to less weed control. Research is necessary to determine specific weed species response to application of reduced rates, either accidental, or by design.

Glufosinate and chlorimuron, although not primarily Photosystem II (PS II) inhibitors, ultimately cause cellular death resulting in a weakened ability to use or disperse light energy. Changes in fluorescence induction (Kautsky curve) have been used extensively in photosynthesis and herbicide research and are the basis for all fluorescence parameters (Abbaspoor and Streibig, 2005; Christensen et al., 2003; Percival and Baker, 1991). The benefits of using fluorescence include its non-invasive procedure, sensitivity to many biotic and abiotic stressors, ease and efficiency and numerous parameters to measure the status of the photosynthetic apparatus (Abbaspoor and Streibig, 2005; Barbagallo et al., 2003; Frankart et al., 2003; Strasser et al., 2000).

Illumination of dark-adapted unstressed leaves produces a rise in chlorophyll fluorescence emission from the ground state ( $F_0$ ) to its maximum value ( $F_m$ ) within one second. An important parameter used in fluorescence research is the  $F_v/F_m$  [ $F_v/F_m = (F_m - F_0)/F_m$ ] parameter (Butler, 1978). The dark adaptation of a leaf allows PSII to be fully reduced at  $Q_A$  and when illuminated the maximum quantum efficiency of the PS II photochemistry can be determined by  $F_v/F_m$ . This parameter is used most often in the literature to indicate plant health with a value of 0.83 indicating no stress to the plant.  $F_v/F_m$  has been used to measure the effect of glyphosate on fluorescence in previous studies but has

not been used to study glufosinate. Kirkwood et al 2000 used this parameter and detected some differences from the control one day after treatment with glyphosate. Olesen and Cedergreen 2010 and Ralph 2000 did not find any effect of glyphosate on Fv/Fm.

The objectives of this research were to evaluate potential interactions among four matrix rate combinations of glufosinate with chlorimuron and thifensulfuron in the greenhouse, determine if the interactions were significant for antagonism and synergism or just additive, determine if results in the greenhouse were consistent with observations in the field, determine if there were differences between visual control and quantitative measurements and, determine if there were different responses among species.

Based on prior research it is expected that, early synergism between herbicides will be observed in some species (14 DAT or before) but at 28 DAT may no longer be evident, the interaction of the two different sulfonylurea herbicides with glufosinate may be similar. Following glufosinate application to weeds, it is expected that fluorescence parameters will indicate injury to the photosynthetic system much earlier than visually observed and changes in fluorescence patterns will be indicative of the interaction between glufosinate and chlorimuron.

## **MATERIALS AND METHODS**

**Field Trial.** Field trials were conducted in 2008 at the Michigan State University Agronomy Research Farm (42°42'42" N, 84°28'13" W) and 2009 at the Michigan

State University Plant Pathology Research Center (42°40'59" N, 84°29'5" W) in East Lansing, MI. The soil at the Agronomy Research Farm was a sandy clay loam with 2.6% organic matter and a pH of 6.3. The soil at the Plant Pathology Research Center was a fine sandy loam with a 2 to 6% slope, a pH of 6.9 and 2.5% organic matter. Fields preparation included fall-plowing followed by cultivation in the spring to obtain maximum weed emergence. The experimental design was a randomized complete block in 2008 and 2009. Treatments differed between years and therefore were separated by year and summarized in Table 10.

Herbicide applications were made using a tractor-mounted compressed-air sprayer calibrated to deliver 178 L / ha at 207 kPa through AirMix 11003 nozzles<sup>a</sup>. Common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.) and giant foxtail (*Setaria faberi* Herm.) were the predominant weed species in both years and were the focus in this study. Other weed species present included; redroot pigweed (*Amaranthus retroflexus* L.), Powell amaranth (*Amaranthus powellii* S. Watson), common ragweed (*Ambrosia artemisiifolia* L.) and large crabgrass (*Digitaria sanguinalis* L. Scop.) in 2008. Weed species in 2009 were similar to those in 2008 but also included a large population of wild mustard.

Visual estimates of weed control were made at 7, 14, 21 and 28 days after treatment (DAT) in 2008 and 7, 14 and 21 DAT in 2009 on a scale of 0% (no control) to 100% (complete control) based on injury compared with the untreated control.

## Greenhouse studies.

*Plant material.* Common lambsquarters, giant foxtail, and velvetleaf from seed, and Canada thistle (*Cirsium arvense* L. Scop.) from root stock<sup>b</sup> were grown in 9-cm pots containing a commercial potting medium<sup>c</sup> in a greenhouse with temperature maintained at  $23 \pm 3^{\circ}\text{C}$ . Natural light was supplemented by high-pressure sodium lamps producing a photosynthetic photon flux density of  $200 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with a photoperiod of 16/8 h light/dark. Pots were watered daily to maintain adequate soil conditions for optimum plant growth. Plants were fertilized with 50 ml of fertilizer solution containing 6 mg/L of 20% nitrogen, 20%  $\text{P}_2\text{O}_5$  and 20%  $\text{K}_2\text{O}$  as needed. Weeds were sprayed at 10-12 cm which is considered larger than optimum size which was done to accentuate differences between herbicide treatments and compensate for greater sensitivity of greenhouse grown plants. There were four replications per experiment and each experiment was repeated four times. Studies with common lambsquarters were repeated six times because of the increased susceptibility to pythium (*Pythium spp.*) and fusarium (*Fusarium spp.*) after glyphosate or glufosinate application.

*Herbicide treatments.* Herbicides were applied in a matrix format so a range of combinations of each herbicide at each rate were used. Glufosinate<sup>d</sup> was applied at rates of 0, 118, 157, 235, and 470 g ai/ha, chlorimuron-ethyl<sup>e</sup> (hereafter referred to as chlorimuron) applied at rates of 0, 2.2, 2.9, 4.4, and 8.8



g ai/ha and thifensulfuron-methyl<sup>f</sup> (hereafter referred to as thifensulfuron) applied at rates of (0, 1.125, 1.5, 2.25, and 4.5 g ai/ha). These rates are representative of 0.0X, 0.25X, 0.33X, 0.5X, and 1X of the label-recommended rate (Anonymous, 2006a; Anonymous, 2006b; Anonymous, 2007). A crop oil concentrate<sup>g</sup> (COC) was included at 1% v/v in treatments containing only chlorimuron or only thifensulfuron. Ammonium sulfate was included at 2% v/v in all treatments containing glufosinate alone or in combination with the sulfonylurea herbicides.

Applications were made using a single-nozzle track sprayer with 8001 even flat fan nozzle<sup>h</sup> calibrated to deliver 187 L/ha at a pressure of 207 kPa. Visual estimates of weed control were taken at 5, 7, 14, 21 and 28 days after treatment (DAT) on a scale of 0% (no control) to 100% (complete control) based on injury compared to the untreated control. Plant height data was collected and above ground biomass harvested and immediately weighed at 28 DAT. Plant samples were oven-dried at 50 C for 48 hr and dry weights were recorded.

*Data analysis.* The data analysis was conducted using ANOVA in the PROC MIXED program in SAS 9.2<sup>i</sup>. Normality of the residuals was evaluated using normal probability and box plots and arc sine data transformations were conducted when there were significant deviations from normality. Homogeneity of variances was evaluated using Levene's test. Herbicide combinations were determined to be antagonistic, synergistic, or additive by comparing the observed plant responses with the expected response when the herbicides are combined. Expected values were calculated using Colby's equation;  $E = X + Y - XY/100$

(Colby, 1967). In the equation, X and Y is the percent growth inhibition by herbicide A and B respectively and E is the expected percent growth inhibition by herbicides A and B combined. Expected and observed responses were compared using Fishers Protected least significant difference (LSD) at  $p = 0.05$  significance. Combinations were determined as antagonistic, synergistic, or additive if the observed response was less than, greater than or similar to the expected response respectively.

### **Fluorescence Studies.**

*Plant material.* Common lambsquarters (*Chenopodium album* L.), were grown from seed in the greenhouse. Approximately 10 seeds per pot were sown into soil media in 900 mL black plastic pots. Plants were thinned to one per pot upon emergence. Canada thistle (*Cirsium arvense* L. Scop.) was grown from root stock obtained in May of 2008 and transplanted into soil media in 900 mL black plastic pots. These plants were genetically similar. Tillers from stock plants were transplanted into fresh media and pots. Canada thistle plants were selected for treatment 2 weeks after transplant.

All plants were grown in greenhouses at Michigan State University campus in East Lansing, MI and experiments took place in May of 2009. Natural light was supplemented by high-pressure sodium lamps that produced a photosynthetic photon flux density of  $200 \text{ mol m}^{-2} \text{ s}^{-1}$ . The photoperiod was 16/8 h light/dark, and the temperature was  $23 \pm 3^\circ\text{C}$ . Pots were watered daily to maintain adequate soil conditions for optimum plant growth. Plants were fertilized with 50 ml of

fertilizer solution containing 6 mg/L of 20% nitrogen, 20% P<sub>2</sub>O<sub>5</sub> and 20% K<sub>2</sub>O as needed. Plants were 10 to 12 cm tall at time of treatment and were randomly assigned to herbicide treatments. Experiments were replicated three times and the experiment repeated three times.

*Herbicide Treatments.* Herbicide treatments consisted of glufosinate and chlorimuron alone and in combinations at the rates given in Table 11. Ammonium sulfate (AMS) at 2% v/v was used as an adjuvant when glufosinate was applied alone or in combination with chlorimuron. Crop oil concentrate was used at 1% v/v for chlorimuron alone. Treatments were applied using a single-tip track sprayer using a TP8001 flat fan nozzle delivering 187 L ha<sup>-1</sup> at a pressure of 207 kPA. Treatments were selected based on preliminary studies to obtain the highest observable interaction, such as, a high and a low rate combined, low rates combined and high rates combined.

*Fluorescence Measurements.* After herbicide application the plants were immediately returned to the greenhouse and prepared for fluorescence evaluation. Fluorescence measurements were taken at 2, 4, 6, 8, 24, 48 and 72 hours after treatment (HAT). The second set of fully emerged leaves above the cotyledons, with at least one more set of fully emerged leaves above were selected for fluorescence evaluation. Leafclips<sup>i</sup> were placed in the middle of the selected leaf directly next to the midvein with the least amount of contact with any major veins. The clip has a small shutter plate that should be closed over the leaf once the clip is attached so that light is excluded and dark adaptation begins to take place. The process of dark adaptation varied depending on plant species,

ambient light history and whether the plant was stressed. The average time required for dark adaptation in this study was 15 minutes. Once dark adapted, the Handy Pocket PEA optical interface was attached to the clip around the shutter plate, the shutter was opened and high intensity LED light passed through a NIR filter, onto the leaf. Then a highly sensitive PIN photodiode detects the fluorescence signal at 10 $\mu$ s intervals for 1 second. The data obtained in the 1 second period was saved in the Handy Pocket PEA<sup>k</sup> and later downloaded into a computer for analysis. Treated plants were visually rated for control, 0 being no control and 100 being complete control, at 7, 14, 21 and 28 days after treatment (DAT). Plant heights were measured at 28 DAT and were then harvested and weighed for fresh weight immediately. Plants harvested were dried at 50°C for 48 hr, dry weights were then determined and samples were discarded.

*Data analysis.* Data were subjected to ANOVA using PROC MIXED in SAS and treatment means for Fv/Fm within species were compared using Fisher's Protected LSD at the  $p = 0.05$  significance level. Data were transformed when necessary for analysis and back-transformed data are presented.

## RESULTS AND DISCUSSION

**Field Studies.** Field studies in 2008 resulted in only one significant interaction; due to the high rate of application of herbicides. Thus a second field study conducted in 2009 included a greater combination or application rates of glufosinate and chlorimuron. Antagonism was observed at low rates of

glufosinate and the range of chlorimuron rates for all species (Table 12). At high rates of glufosinate the antagonism was no longer observed except when applied in combination with 0.5X chlorimuron on velvetleaf 7 DAT (Table 12). Synergism was observed between high rates of glufosinate and the range of chlorimuron rates for all other species (Table 12).

**Greenhouse Studies.** The hypothesis was that early synergism between herbicides would be evident in some species, but by 28 DAT the synergism may no longer be evident. An example of when the interaction was synergistic early was the combination of chlorimuron and glufosinate applied to common lambsquarters. Visual observations 7 DAT confirmed synergism in combinations at all rates of glufosinate with various chlorimuron rates (Figure 18a). This synergism was apparent with only one combination at 28 DAT while other combinations showed additive effect (Figure 18b). An example of synergism not seen early but was evident at 28 DAT visual observations. One example of this was the effect of the combination of chlorimuron and glufosinate on giant foxtail. At the 1/4X rate of glufosinate combined with the range of chlorimuron rates, either an additive effect or antagonism at 7 DAT was evident which became synergistic later (Figure 19). Another example of synergism found at 28 DAT was the effect of the combination of thifensulfuron and glufosinate on velvetleaf. At 7 DAT this combination showed some synergistic results along with an additive effect and antagonism (Figure 20a), by 28 DAT however all combinations with less than 1X glufosinate resulted in synergism (Figure 20b). The combination of thifensulfuron and glufosinate on giant foxtail resulted in similar synergism at 28

DAT but was only evident in the 1/4X glufosinate combination (data not shown). Early synergism that results in antagonism over time was not always seen between combinations but there were examples of late antagonism (Figure 19, 20). There were combinations on some species that resulted in late synergism (data not shown).

The hypothesis that the two sulfonylurea herbicides individually in combination with glufosinate would act similarly was not always supported (data not shown). Again, this was species dependent and combination dependent. An example of when the sulfonylurea herbicides produced similar responses was in combinations with glufosinate on giant foxtail. Although there were a few examples of when the sulfonylurea herbicides produced similar results, more often where they were dissimilar for weed control. The combination of the sulfonylurea herbicides and glufosinate resulted in more significant interactions in the thifensulfuron combination with glufosinate than the chlorimuron combination with glufosinate on Canada thistle, velvetleaf and common lambsquarters (data not shown).

**Fluorescence Studies.** Fv/Fm for Canada thistle was chosen as the parameter to examine for significant differences between herbicide combinations. The interactions between herbicides are discussed in great detail for both Canada thistle and common lambsquarters in Appendix B. The Fv/Fm of a healthy plant is 0.83, a value smaller than 0.83 is an indication of the inability of the plant, due to injury, to efficiently photosynthesize.

The Fv/Fm for plants receiving the combination of 0.25X chlorimuron + 0.25X glufosinate and 1X chlorimuron + 0.25X glufosinate at 2 HAT were significantly higher than those receiving glufosinate alone (Figure 21a,b). This remained consistent for all readings until 24 HAT when they no longer become significantly different. The 1X chlorimuron + 0.25X glufosinate combination was not significantly different from the glufosinate alone at 72 HAT but the combination of 0.25X chlorimuron + 0.25X glufosinate was significantly lower than of glufosinate applied alone at 72 HAT (Figure 21a). Fv/Fm values at 2 HAT for plants sprayed with glufosinate + chlorimuron showed significant differences from the control. (Figure 22a). The Fv/Fm values at 72 HAT following the 0.25X glufosinate and 0.25X chlorimuron combination were significantly lower than that of the 1X glufosinate + 1X chlorimuron combination (data not shown). Fv/Fm values of Canada thistle following combinations of glufosinate and chlorimuron were not significantly different from the Fv/Fm after glufosinate alone application (Figure 22b).

In summary early synergism was observed in interactions with glufosinate in the herbicide combination due to the earlier observable (sometimes by 3 days) injury in comparison to combinations with the sulfonylurea herbicides (more than 7 days). After that time the interaction between the herbicides became more apparent as synergism, antagonism or additive effect which was dependent on species and the rate of the combination. There were similarities between field and greenhouse studies; however, the interactions were not always the same due to differences in the stage of weed at time of herbicide application in the

field. Fluorescence measurements did not show differences between glufosinate and the combination with chlorimuron. However, glufosinate rapidly acted to break down the photosynthetic system 2 HAT and chlorimuron activity was not seen in fluorescence measurements taken up to 72 HAT.



## **SOURCE OF MATERIALS**

<sup>a</sup>AirMix 11003, Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434.

<sup>b</sup>Don Penner's Farm Williamston, MI. pennerd@msu.edu.

<sup>c</sup>Baccto® High Porosity Professional Potting Mix, Michigan Peat Co., Houston, TX.

<sup>d</sup>Liberty®, Bayer CropScience, Research Triangle Park, NC.

<sup>e</sup>CLASSIC®, DuPont Agricultural Products, Newark, DE.

<sup>f</sup>HARMONY®, DuPont Agricultural Products, Newark, DE.

<sup>g</sup>Crop oil concentrate. Loveland Products, Inc. PO Box 1286 Greely, CO 80632.

<sup>h</sup>TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

<sup>i</sup>The SAS System for Windows Version 9.2, SAS Institute Inc, 100 SAS Campus Dr., Cary, NC 275.

<sup>j</sup>Leaf Clips, Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn, Norfolk, U.K.

<sup>k</sup>Handy Pocket PEA, Hansatech Instruments, Narborough Road, King's Lynn Norfolk, U.K.

*Table 10.* Herbicide combinations applied in field studies  
2008 and 2009.<sup>a</sup>

<b>Herbicides Applied by Year - Field Studies</b>	
<b>2008</b>	<b>2009</b>
Control	Control
1X GLU <sup>b</sup>	0.25X GLU
1X CHL	0.5X GLU
0.5X GLU + 0.5X CHL	1X GLU
0.5X GLU + 1X CHL	0.25X CHL
1X GLU+ 0.5X CHL	0.5X CHL
1X GLU + 1X CHL	1X CHL
1X THI	0.25X GLU + 0.25X CHL
	0.25X GLU + 0.5X CHL
	0.25X GLU + 1X CHL
	0.5X GLU + 0.25X CHL

<sup>a</sup>Where 1X = the labeled rate; Glufosinate applied as LIBERTY 1X = 420 g ai/ha; Chlorimuron applied as CLASSIC 1X = 8.8 g ai/ha; Thifensulfuron applied as HARMONY DS 1X = 4.5 g ai/ha.

<sup>b</sup>Abbreviations: GLU, Glufosinate; CHL, Chlorimuron; THI, Thifensulfuron.

**Table 11.** Herbicide combinations applied in fluorescence studies.<sup>a</sup>

<b>Herbicides Applied by Species - Fluorescence Studies</b>	
<b>CIRAR + CHEAL</b>	<b>ABUTH + SETFA</b>
Control	Control
0.25X GLU <sup>b</sup>	0.25X GLU
0.5X GLU	0.5X GLU
1X GLU	1X GLU
0.25X CHL	1X CHL
0.5X CHL	0.25X GLU + 1X CHL
1X CHL	0.25X GLU + 1X GLY
0.25X GLU + 0.25X CHL	
0.25X GLU + 1X CHL	
0.5X GLU + 0.5X CHL	
1X GLU + 0.25X CHL	
1X GLU + 0.5X CHL	
1X GLU + 1X CHL	

<sup>a</sup> Where 1X = the labeled rate; glufosinate applied as LIBERTY 1X = 420 g ai/ha; chlorimuron applied as CLASSIC 1X = 8.8 g ai/ha.

<sup>b</sup> Abbreviations: GLU, glufosinate; CHL, chlorimuron.

**Table 12.** Visual control 7 and 28 days after treatment of velvetleaf, common lambsquarters and giant foxtail when applied with combinations of glufosinate and chlorimuron in 2009 field study.<sup>a</sup>

Herbicide Rate <sup>b,c,d</sup>		ABUTH				CHEAL				SETFA			
		7 DAT		28 DAT		7 DAT		28 DAT		7 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
GLU	CHL	g ai/ha											
		-----%											
0	0	-	0	-	0	-	0	-	0	-	0	-	0
118	0	-	30	-	31	-	35	-	31	-	49	-	76
235	0	-	48	-	39	-	46	-	29	-	64	-	73
470	0	-	100	-	70	-	97	-	89	-	94	-	86
0	2.2	-	43	-	29	-	16	-	30	-	6	-	35
0	4.4	-	26	-	39	-	15	-	44	-	2	-	51
0	8.8	-	39	-	38	-	23	-	25	-	25	-	46
118	2.2	100	44 (-)	80	43 (-)	98	32 (-)	92	33 (-)	94	56 (-)	91	78 (-)
118	4.4	100	29 (-)	80	69 (-)	98	36 (-)	93	48 (-)	94	58 (-)	93	73 (-)
118	8.8	100	39 (-)	80	83	98	21 (-)	91	50 (-)	95	79 (-)	93	80 (-)
235	2.2	70	29 (-)	56	71 (+)	55	20 (-)	50	86 (+)	66	73 (+)	82	91 (+)
235	4.4	61	28 (-)	62	95 (+)	54	54	60	41 (-)	65	81 (+)	87	81 (-)
235	8.8	68	94 (+)	61	98 (+)	59	42 (-)	47	44	73	78	85	86
470	4.4	49	14 (-)	58	97 (+)	45	84 (+)	61	86 (+)	50	74 (+)	88	90
470	8.8	58	93 (+)	57	95 (+)	50	48	49	84 (+)	61	89 (+)	87	93 (+)
LSD (0.05) <sup>e</sup>		3		2		2		2		2		2	

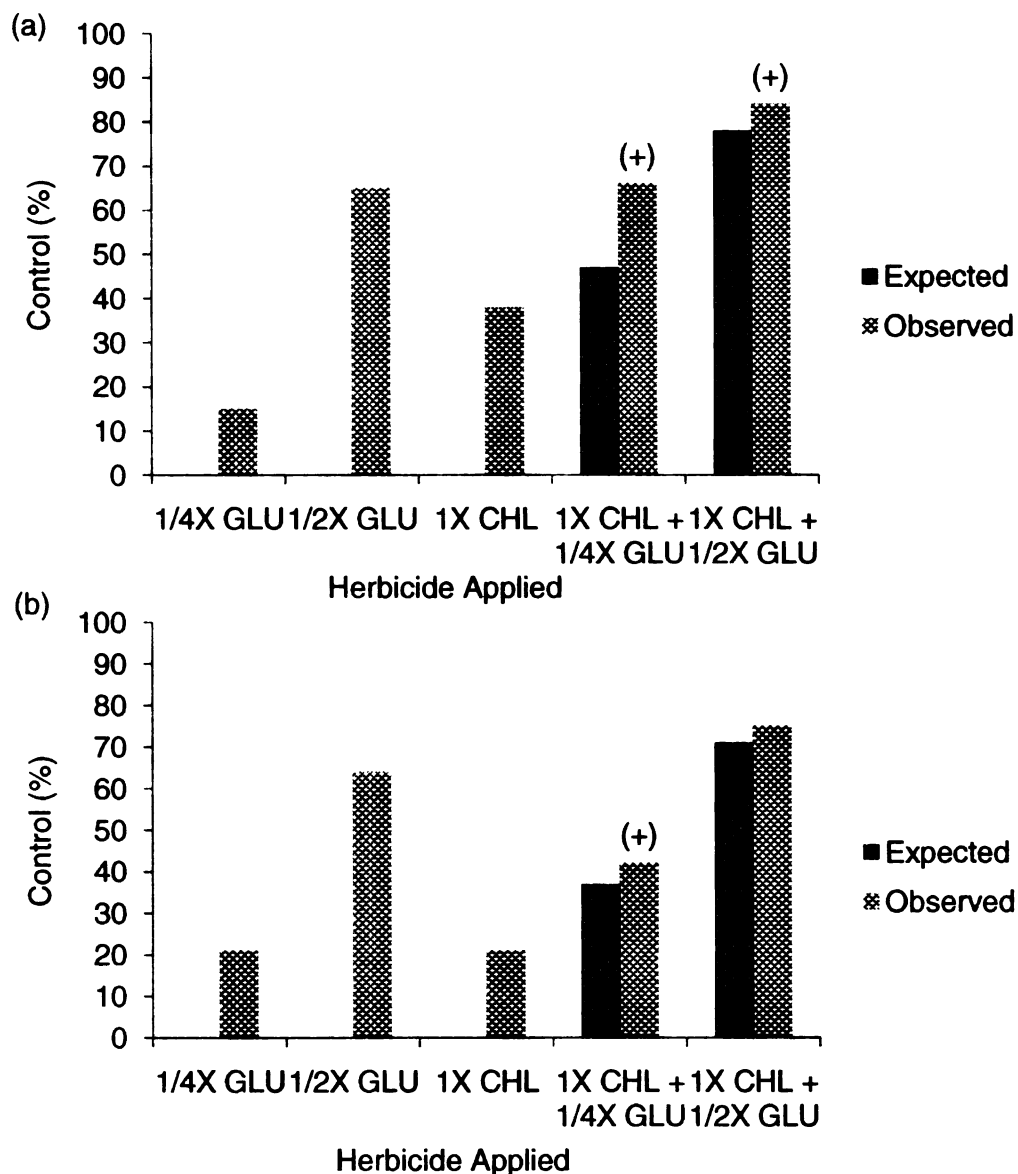
<sup>a</sup> + and - denote a synergistic and antagonistic interaction for a given combination according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: GLU, glufosinate; CHL, chlorimuron; ABUTH, velvetleaf; CHEAL, common lambsquarters; SETFA, giant foxtail; DAT, days after treatment; Exp., expected; Obs., observed.

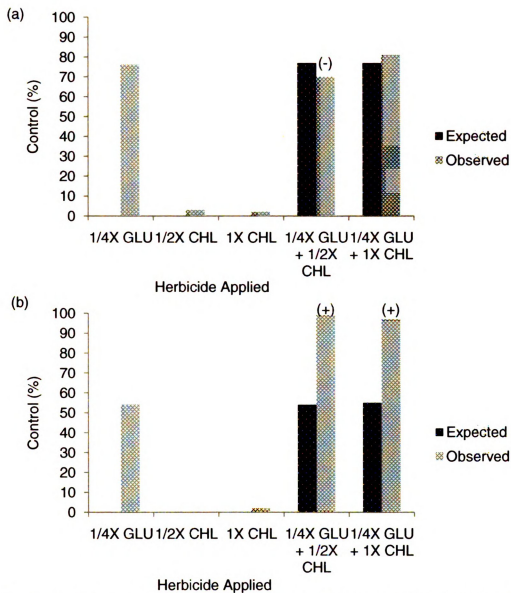
<sup>c</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing only chlorimuron also included crop oil concentrate at 1% v/v.

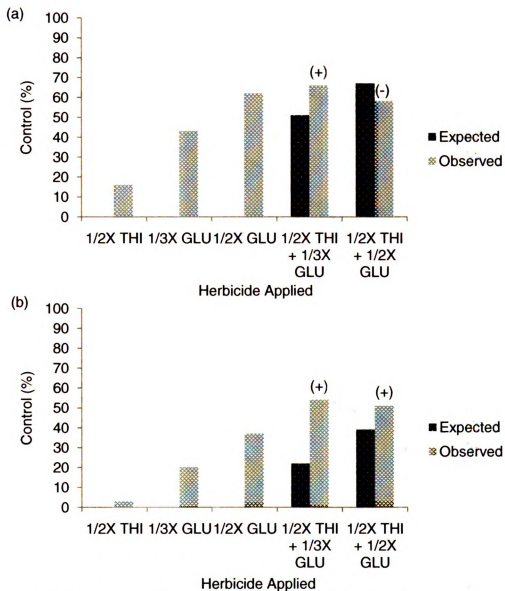
<sup>e</sup> LSD values may be used to compare values.



**Figure 18.** Chlorimuron(CHL) and glufosinate(GLU) on common lambsquarters visual observations in the greenhouse (a) 7 days after treatment and (b) 28 days after treatment. Synergism by Colby's method indicated by a (+). LSD = 5 for 7 DAT and LSD = 12 for 28 DAT.



**Figure 19.** Chlorimuron(CHL) and glufosinate(GLU) on giant foxtail visual observations in the greenhouse (a) 7 days after treatment and (b) 28 days after treatment. Antagonism and synergism by Colby's method indicated by a (-) or (+) respectively. LSD = 5 for 7 DAT and LSD = 8 for 28 DAT.



**Figure 20.** Thifensulfuron (THI) and glufosinate (GLU) on velvetleaf visual observations in the greenhouse (a) 7 days after treatment and (b) 28 days after treatment. Antagonism and synergism by Colby's method indicated by a (-) or (+) respectively. LSD = 5 for 7 DAT and LSD = 8 for 28 DAT.

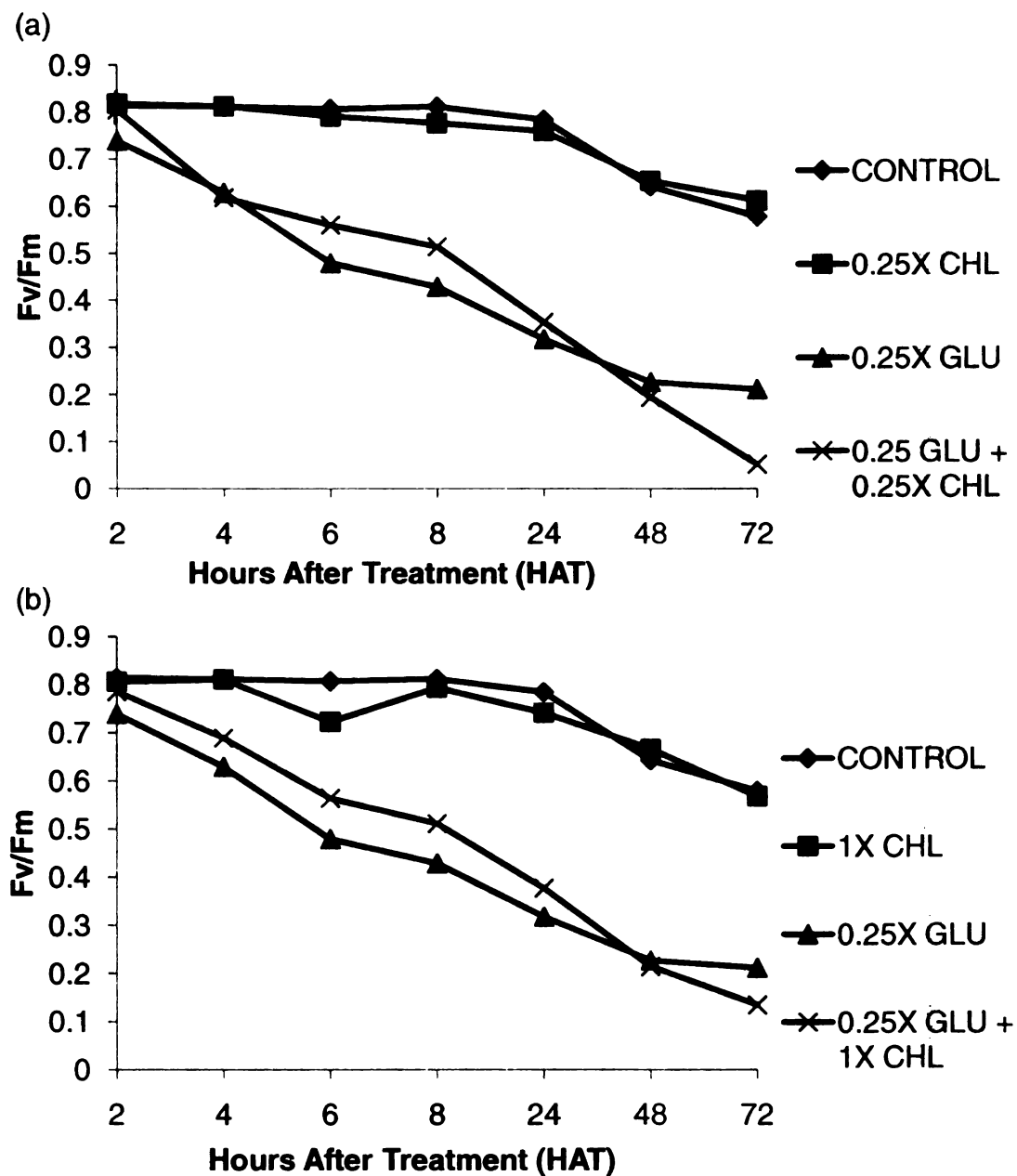


Figure 21 . Fv/Fm of glufosinate(GLU) and chlorimuron(CHL) combinations on Canada thistle. Where a (\*) indicates the combination is significantly different from glufosinate applied alone.



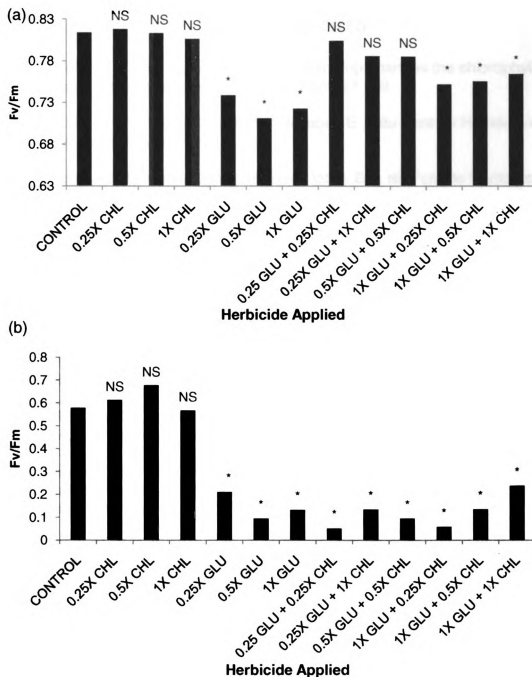


Figure 22. Fv/Fm of glufosinate (GLU) and chlorimuron (CHL) on Canada thistle at (a) 2 hours after treatment and (b) 72 hours after treatment. Where a (\*) indicates the combination is significantly different from the control.

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## **APPENDIX**

**Table 13.** Visual control 7 and 28 days after treatment of velvetleaf, common lambsquarters and giant foxtail when applied with combinations of glyphosate, glufosinate and chlorimuron in 2009 field study.<sup>a</sup>

			Visual Control											
			ABUTH				CHEAL				SETFA			
Herbicide Rate <sup>b,c,d</sup>			7 DAT		28 DAT		7 DAT		28 DAT		7 DAT		28 DAT	
GLY	GLU	CHL	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ae/ha	g ai/ha		-----%											
0	0	0	-	0	-	0	-	0	-	0	-	0	-	0
210	0	0	-	38	-	46	-	36	-	48	-	75	-	86
420	0	0	-	48	-	100	-	29	-	85	-	89	-	99
840	0	0	-	48	-	100	-	48	-	99	-	80	-	100
0	118	0	-	30	-	31	-	35	-	31	-	49	-	76
0	235	0	-	48	-	39	-	46	-	29	-	64	-	73
0	470	0	-	100	-	70	-	97	-	89	-	94	-	86
0	0	2.2	-	43	-	29	-	16	-	30	-	6	-	35
0	0	4.4	-	26	-	39	-	15	-	44	-	2	-	51
0	0	8.8	-	39	-	38	-	23	-	25	-	25	-	46
210	470	0	100	99	85	63 (-)	98	95	94	89	98	95	98	95
420	235	0	73	99 (+)	100	100	62	95 (+)	89	100 (+)	96	91	100	86 (-)
840	118	0	64	95 (+)	100	90 (-)	66	95 (+)	99	91 (-)	90	95	100	89 (-)
840	470	0	64	90 (+)	100	100	66	93 (+)	99	100	90	90	100	100
210	0	2.2	64	42 (-)	62	78 (+)	47	36 (-)	63	71 (+)	77	91 (+)	91	94
210	0	4.4	54	26 (-)	67	59 (-)	46	37 (-)	71	72	76	86 (+)	94	94
210	0	8.8	61	81 (+)	68	98 (+)	51	55	61	95 (+)	81	71 (+)	93	94
420	0	2.2	70	92 (+)	100	90 (-)	40	83 (+)	89	86	90	90	99	100
420	0	4.4	61	29 (-)	100	95	39	91 (+)	92	81 (-)	89	90	99	98
420	0	8.8	67	38 (-)	100	100	45	57 (+)	89	72 (+)	92	86	99	100
840	0	4.4	61	61	100	99	55	36 (-)	99	83 (-)	81	89 (-)	100	100
840	0	8.8	67	92 (+)	100	100	59	60	99	98	85	91	100	100
0	118	2.2	100	44 (-)	80	43 (-)	98	32 (-)	92	33 (-)	94	56 (-)	91	78 (-)
0	118	4.4	100	29 (-)	80	69 (-)	98	36 (-)	93	48 (-)	94	58 (-)	93	73 (-)
0	118	8.8	100	39 (-)	80	83	98	21 (-)	91	50 (-)	95	79 (-)	93	80 (-)
0	235	2.2	70	29 (-)	56	71 (+)	55	20 (-)	50	86 (+)	66	73 (+)	82	91 (+)
0	235	4.4	61	28 (-)	62	95 (+)	54	54	60	41 (-)	65	81 (+)	87	81 (-)
0	235	8.8	68	94 (+)	61	98 (+)	59	42 (-)	47	44	73	78	85	86
0	470	4.4	49	14 (-)	58	97 (+)	45	84 (+)	61	86 (+)	50	74 (+)	88	90
0	470	8.8	58	93 (+)	57	95 (+)	50	48	49	84 (+)	61	89 (+)	87	93 (+)
LSD (0.05) <sup>e</sup>			3		2		2		2		2		2	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for a given combination according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: GLY, glyphosate; GLU, glufosinate; CHL, chlorimuron; ABUTH, velvetleaf; CHEAL, common lambsquarters; SETFA, giant foxtail; DAT, days after treatment; Exp.,

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2%

<sup>d</sup> Treatments containing only chlorimuron or thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 14.** Percent control compared to the untreated for combinations of glyphosate and glufosinate on Canada thistle 5, 7, 14, 21 and 28 days after treatment.<sup>a</sup>

		Visual Control - Canada thistle							
Herbicide Rate <sup>c</sup>		5 DAT <sup>b</sup>		7 DAT		14 DAT		21 DAT	
Glyphosate	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ae/ha	g ai/ha	-----%-----							
0	0	-	0	-	0	-	0	-	0
210	0	-	33	-	59	-	90	-	96
280	0	-	31	-	76	-	86	-	100
420	0	-	43	-	75	-	94	-	99
840	0	-	51	-	75	-	95	-	100
0	118	-	50	-	58	-	34	-	39
0	157	-	63	-	71	-	83	-	90
0	235	-	64	-	79	-	83	-	86
0	470	-	68	-	75	-	94	-	98
210	118	67	55 (-)	82	60 (-)	93	45 (-)	98	58
280	118	66	55 (-)	91	58 (-)	91	49 (-)	100	64
420	118	74	45 (-)	90	54 (-)	96	56 (-)	100	62
840	118	77	44 (-)	89	59 (-)	97	64 (-)	100	74
210	157	74	48 (-)	88	55 (-)	99	61 (-)	100	61
280	157	73	41 (-)	94	51 (-)	99	58 (-)	100	65
420	157	78	45 (-)	93	53 (-)	99	58 (-)	100	59
840	157	82	50 (-)	93	61 (-)	99	66 (-)	100	78
210	235	77	65 (-)	90	69 (-)	98	77 (-)	99	72
280	235	76	65 (-)	96	74 (-)	99	70 (-)	100	86
420	235	81	65 (-)	95	69 (-)	99	64 (-)	100	81
840	235	84	61 (-)	95	65 (-)	99	84 (-)	100	78
210	470	77	80	89	76 (-)	99	92	100	100
280	470	76	80	94	83 (-)	99	98	100	95
420	470	81	80	94	75 (-)	100	94	100	100
840	470	85	73 (-)	94	80 (-)	100	94	100	96
LSD (0.05) <sup>d</sup>		9		8		10		12	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs.,

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> LSD values may be used to compare values.

**Table 15.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of glyphosate and glufosinate for canada thistle and velvetleaf.<sup>a</sup>

Herbicide Rate <sup>c</sup>		CIRAR <sup>b</sup>				ABUTH		
		FWR		HR		FWR		HR
Glyphosate	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
g ae/ha	g ai/ha	% Reduction						
0	0	-	0	-	0	-	0	-
210	0	-	95	-	81	-	85	-
280	0	-	92	-	83	-	86	-
420	0	-	95	-	86	-	94	-
840	0	-	97	-	86	-	93	-
0	118	-	53	-	43	-	65	-
0	157	-	86	-	83	-	80	-
0	235	-	89	-	74	-	84	-
0	470	-	83	-	88	-	94	-
210	118	98	57 (-)	89	53 (-)	95	77 (-)	92
280	118	97	74 (-)	90	55 (-)	95	84 (-)	91
420	118	98	66 (-)	91	67 (-)	98	79 (-)	94
840	118	98	64 (-)	91	64 (-)	98	84 (-)	93
210	157	99	57 (-)	96	57 (-)	97	83 (-)	95
280	157	99	62 (-)	97	58 (-)	97	71 (-)	94
420	157	99	69 (-)	98	48 (-)	99	80 (-)	96
840	157	99	74 (-)	98	67 (-)	99	67 (-)	96
210	235	99	77 (-)	94	73 (-)	97	91	96
280	235	100	78 (-)	96	64 (-)	98	80 (-)	96
420	235	100	71 (-)	96	64 (-)	99	80 (-)	97
840	235	100	87 (-)	96	62 (-)	99	80 (-)	96
210	470	99	89	98	83 (-)	99	91	98
280	470	99	97	98	89	99	80 (-)	97
420	470	99	94	98	84 (-)	100	88 (-)	98
840	470	99	92	98	80 (-)	100	89 (-)	98
LSD (0.05) <sup>d</sup>		11		12		11		12

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CIRAR, canada thistle; ABUTH, velvetleaf; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> LSD values may be used to compare values.

**Table 16.** Percent control compared to the untreated for combinations of glyphosate and glufosinate on velvetleaf 7, 14, 21 and 28 days after treatment.<sup>a</sup>

		Visual Control - Velvetleaf							
Herbicide Rate <sup>c</sup>		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
Glyphosate	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ae/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
210	0	-	43	-	73	-	78	-	86
280	0	-	44	-	80	-	85	-	87
420	0	-	49	-	96	-	96	-	99
840	0	-	54	-	98	-	100	-	100
0	118	-	58	-	13	-	10	-	30
0	157	-	75	-	41	-	23	-	38
0	235	-	81	-	44	-	28	-	53
0	470	-	85	-	95	-	94	-	81
210	118	76	68 (-)	76	35 (-)	80	36 (-)	91	54
280	118	76	62 (-)	82	53 (-)	87	36 (-)	91	54
420	118	77	59 (-)	97	54 (-)	97	50 (-)	99	66
840	118	79	63 (-)	98	76 (-)	100	56 (-)	100	68
210	157	86	57 (-)	84	33 (-)	82	38 (-)	91	56
280	157	86	56 (-)	88	46 (-)	88	39 (-)	91	57
420	157	86	63 (-)	98	60 (-)	97	39 (-)	99	60
840	157	88	64 (-)	98	58 (-)	100	53 (-)	100	67
210	235	89	77 (-)	85	73 (-)	84	46 (-)	94	65
280	235	89	76 (-)	89	39 (-)	89	43 (-)	94	58
420	235	90	79 (-)	98	50 (-)	97	43 (-)	99	57
840	235	91	70 (-)	98	44 (-)	100	49 (-)	100	56
210	470	91	81 (-)	99	84 (-)	99	68 (-)	97	73
280	470	92	82 (-)	99	49 (-)	99	43 (-)	98	59
420	470	92	83 (-)	100	73 (-)	100	66 (-)	99	74
840	470	93	81 (-)	100	88 (-)	100	88 (-)	100	74
LSD (0.05) <sup>d</sup>		5		11		12		10	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> LSD values may be used to compare values.



**Table 17.** Percent control compared to the untreated for combinations of glyphosate and glufosinate on common lambsquarters 5, 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c</sup>		Visual Control - Common lambsquarters							
		5 DAT <sup>b</sup>		7 DAT		14 DAT		21 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Glyphosate	Glufosinate	-----%							
g ae/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
210	0	-	0	-	10	-	26	-	49
280	0	-	3	-	47	-	58	-	81
420	0	-	16	-	46	-	67	-	93
840	0	-	48	-	63	-	91	-	96
0	118	-	0	-	12	-	13	-	18
0	157	-	35	-	64	-	52	-	51
0	235	-	45	-	75	-	50	-	66
0	470	-	50	-	74	-	74	-	100
210	118	4	24 (+)	21	22	36	47	56	38
280	118	8	24 (+)	53	36	66	51	85	57
420	118	21	29	52	34 (-)	73	44 (-)	93	48
840	118	48	36 (-)	68	59	91	71 (-)	96	74
210	157	36	0 (-)	68	15 (-)	66	26 (-)	73	34
280	157	39	49 (+)	78	48 (-)	78	42 (-)	88	43
420	157	48	50	81	48 (-)	85	59 (-)	95	64
840	157	65	45 (-)	86	40 (-)	94	56 (-)	97	65
210	235	46	49	77	51 (-)	64	39 (-)	85	54
280	235	48	53	84	69	78	54 (-)	90	61
420	235	55	46 (-)	88	70 (-)	85	62 (-)	99	67
840	235	70	60 (-)	92	66 (-)	97	72 (-)	99	68
210	470	47	65 (+)	76	82	78	67	100	73
280	470	50	65 (+)	89	84	84	80	100	86
420	470	57	64	86	87	87	90	100	97
840	470	68	67	90	87	99	93	100	99
LSD (0.05) <sup>d</sup>		9		17		17		16	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs.,

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> LSD values may be used to compare values.

**Table 18.** Percent control compared to the untreated for combinations of glyphosate and glufosinate on giant foxtail 5, 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c</sup>		Visual Control - Giant foxtail							
		5 DAT <sup>b</sup>		7 DAT		14 DAT		21 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Glyphosate	Glufosinate	%-----							
g ae/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
210	0	-	5	-	39	-	65	-	74
280	0	-	1	-	34	-	71	-	84
420	0	-	9	-	66	-	78	-	99
840	0	-	8	-	48	-	88	-	99
0	118	-	21	-	84	-	40	-	33
0	157	-	48	-	88	-	64	-	65
0	235	-	53	-	83	-	80	-	70
0	470	-	61	-	96	-	96	-	78
210	118	25	46 (+)	91	95	80	73	84	66 (-)
280	118	22	38 (+)	89	95	83	54 (-)	91	60 (-)
420	118	28	43 (+)	96	93	87	55 (-)	99	65 (-)
840	118	27	46 (+)	90	95	93	74 (-)	99	89
210	157	50	44	92	95	88	56 (-)	91	63 (-)
280	157	48	49	92	95	89	73 (-)	95	64 (-)
420	157	52	46	93	88	92	58 (-)	99	60 (-)
840	157	51	46	92	95	96	76 (-)	100	80 (-)
210	235	55	63	89	95	94	81 (-)	93	74 (-)
280	235	53	63 (+)	88	96	94	85 (-)	96	79 (-)
420	235	57	53	95	95	96	83 (-)	100	71 (-)
840	235	56	48 (-)	97	94	97	79 (-)	100	76 (-)
210	470	63	54 (-)	98	96	99	98	91	91
280	470	62	63	98	95	99	90	96	94
420	470	65	65	99	95	99	94	99	93
840	470	64	68	98	99	100	95	100	99
LSD (0.05) <sup>d</sup>		8		14		9		17	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> LSD values may be used to compare values.

**Table 19.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of glyphosate and glufosinate for common lambsquarters and giant foxtail.<sup>a</sup>

Herbicide Rate <sup>c</sup>		CHEAL <sup>b</sup>				SETFA			
Glyphosate	Glufosinate	FWR		HR		FWR		HR	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ae/ha	g ai/ha	% Reduction							
0	0	-	0	-	0	-	0	-	0
210	0	-	25	-	28	-	98	-	86
280	0	-	42	-	43	-	99	-	96
420	0	-	66	-	65	-	99	-	88
840	0	-	72	-	83	-	99	-	97
0	118	-	31	-	28	-	90	-	84
0	157	-	38	-	32	-	93	-	80
0	235	-	37	-	39	-	100	-	97
0	470	-	63	-	56	-	99	-	97
210	118	45	39	49	33	100	97	99	83
280	118	54	49	57	39	100	100	99	97
420	118	71	38 (-)	76	28 (-)	100	98	99	90
840	118	76	52 (-)	87	55 (-)	100	100	99	95
210	157	51	27 (-)	48	22 (-)	100	100	98	97
280	157	54	46	52	23 (-)	100	100	99	98
420	157	73	51 (-)	71	46 (-)	100	96	99	91
840	157	78	51 (-)	88	43 (-)	100	99	99	94
210	235	53	49	57	37 (-)	100	100	99	97
280	235	56	47	58	38 (-)	100	100	100	98
420	235	74	53	75	43 (-)	100	100	99	96
840	235	80	52 (-)	90	50 (-)	100	100	100	98
210	470	71	70	60	59	100	100	100	98
280	470	73	62	61	54	100	100	100	97
420	470	84	70	79	61 (-)	100	100	100	98
840	470	90	70	91	69 (-)	100	100	100	98
LSD (0.05) <sup>d</sup>		12		18		5		9	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CHEAL, common lambsquarters; SETFA, giant foxtail; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate and/or glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> LSD values may be used to compare values.

**Table 20.** Percent control compared to the untreated for combinations of glyphosate and chlorimuron on Canada thistle 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Canada thistle							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Chlorimuron	Glyphosate	-----%							
g ai/ha	g ae/ha								
0	0	-	0	-	0	-	0	-	0
2.2	0	-	8	-	25	-	26	-	43
2.93	0	-	26	-	50	-	45	-	53
4.4	0	-	31	-	38	-	50	-	60
8.8	0	-	38	-	48	-	54	-	71
0	210	-	68	-	75	-	90	-	89
0	280	-	54	-	68	-	85	-	83
0	420	-	59	-	78	-	94	-	91
0	840	-	85	-	95	-	98	-	99
2.2	210	69	53 (-)	80	68 (-)	92	78 (-)	93	80
2.93	210	76	76	88	85	94	94	95	94
4.4	210	78	70	85	88	95	91	96	91
8.8	210	80	71 (-)	87	90	96	95	97	96
2.2	280	57	71 (-)	75	85 (+)	88	94 (+)	89	95
2.93	280	66	83 (+)	84	93	91	100 (+)	91	100
4.4	280	68	66	80	85	92	93	93	96
8.8	280	71	69	83	85	93	90	96	96
2.2	420	62	85 (+)	83	100 (+)	95	100	95	100
2.93	420	69	75	89	93	97	100	96	100
4.4	420	72	79	86	98 (+)	97	98	97	100
8.8	420	75	85 (+)	88	98 (+)	97	100	98	100
2.2	840	86	90	95	100	98	100	99	100
2.93	840	89	88	98	95	99	100	99	100
4.4	840	90	89	97	100	99	100	100	100
8.8	840	91	89	98	100	99	100	100	100
LSD (0.05) <sup>e</sup>		9		9		7		7	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 21.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of chlorimuron and glyphosate for canada thistle and velvetleaf.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CIRAR <sup>b</sup>				ABUTH			
		FWR		HR		FWR		HR	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Chlorimuron	Glyphosate	% Reduction							
g ai/ha	g ae/ha								
0	0	-	0	-	0	-	0	-	0
2.2	0	-	36	-	61	-	42	-	50
2.93	0	-	59	-	79	-	66	-	62
4.4	0	-	63	-	81	-	46	-	54
8.8	0	-	79	-	86	-	64	-	68
0	210	-	86	-	86	-	51	-	67
0	280	-	80	-	82	-	59	-	74
0	420	-	92	-	85	-	66	-	76
0	840	-	97	-	89	-	83	-	81
2.2	210	90	86	93	83 (-)	67	68	81	63 (-)
2.93	210	92	94	97	91	82	73	86	77 (-)
4.4	210	95	94	97	88 (-)	69	77	84	77
8.8	210	98	96	98	87 (-)	81	77	89	79 (-)
2.2	280	86	95	92	89	74	75	86	79
2.93	280	90	97	96	91	84	75	89	75 (-)
4.4	280	92	92	96	88 (-)	75	79	88	75 (-)
8.8	280	97	95	98	86 (-)	82	78	91	75 (-)
2.2	420	95	98	94	94	80	79	87	77 (-)
2.93	420	97	98	97	89 (-)	89	80	90	77 (-)
4.4	420	97	97	97	91	82	77	88	76 (-)
8.8	420	99	97	98	90 (-)	88	78	92	76 (-)
2.2	840	98	98	96	93	89	82	90	77 (-)
2.93	840	98	97	98	91	93	92	91	83
4.4	840	99	97	98	93	89	80	90	77 (-)
8.8	840	99	98	98	92	93	87	93	80
LSD (0.05) <sup>e</sup>		10		8		13		9	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CIRAR, canada thistle; ABUTH, velvetleaf; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 22.** Percent control compared to the untreated for combinations of glyphosate and chlorimuron on velvetleaf 7, 14, 21 and 28 days after treatment.<sup>a</sup>

		Visual Control - Velvetleaf							
Herbicide Rate <sup>c,d</sup>		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
Chlorimuron	Glyphosate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ae/ha	-----%							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	44	-	46	-	44	-	46
2.93	0	-	66	-	62	-	58	-	61
4.4	0	-	51	-	51	-	48	-	50
8.8	0	-	52	-	56	-	62	-	68
0	210	-	34	-	46	-	64	-	49
0	280	-	40	-	53	-	64	-	70
0	420	-	43	-	58	-	70	-	69
0	840	-	51	-	66	-	89	-	94
2.2	210	63	56	66	59	78	67 (-)	71	69
2.93	210	78	65	77	69	86	85	80	79
4.4	210	70	61	70	69	82	85	75	80
8.8	210	70	62	73	67	88	81	85	82
2.2	280	66	59	72	68	78	80	81	85
2.93	280	79	61 (-)	79	65 (-)	82	78	86	77
4.4	280	71	58	73	62 (-)	79	82	82	81
8.8	280	71	63	76	65	84	85	88	79
2.2	420	68	63	74	76	84	89	81	87
2.93	420	80	66 (-)	81	74	88	88	88	87
4.4	420	72	63	75	74	84	88	83	90
8.8	420	73	57 (-)	77	71	89	88	89	91
2.2	840	73	68	80	74	96	90	97	93
2.93	840	84	66 (-)	85	86	97	96	98	98
4.4	840	78	64 (-)	80	72	96	88	97	88
8.8	840	78	74	82	75	98	92	98	95
LSD (0.05) <sup>e</sup>		14		11		11		11	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 23.** Percent control compared to the untreated for combinations of glyphosate and chlorimuron on common lambsquarters 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Common lambsquarters							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Chlorimuron	Glyphosate	%							
g ai/ha	g ae/ha								
0	0	-	0	-	0	-	0	-	0
2.2	0	-	13	-	6	-	11	-	8
2.93	0	-	11	-	15	-	17	-	16
4.4	0	-	19	-	20	-	21	-	21
8.8	0	-	24	-	28	-	25	-	25
0	210	-	31	-	44	-	41	-	41
0	280	-	34	-	51	-	38	-	51
0	420	-	57	-	69	-	75	-	77
0	840	-	70	-	81	-	88	-	93
2.2	210	39	43	47	63	49	57	49	73
2.93	210	38	34	52	50	50	56	47	68
4.4	210	43	42	55	63	54	69	52	71
8.8	210	46	50	59	56	55	63	52	76
2.2	280	42	57	53	68	45	68 (+)	54	71
2.93	280	41	38	58	48 (-)	47	45	54	65
4.4	280	46	40	61	60	51	61	59	71
8.8	280	49	55	64	68	53	73 (+)	62	81
2.2	420	62	73	70	84	76	84	77	90
2.93	420	62	78 (+)	74	84	78	88	78	91
4.4	420	64	82 (+)	75	87	80	88	81	90
8.8	420	66	70	78	79	82	84	83	88
2.2	840	73	76	82	84	88	91	93	96
2.93	840	73	79	84	86	89	93	93	85
4.4	840	75	82	85	88	90	94	93	97
8.8	840	76	79	87	96	91	97	95	99
LSD (0.05) <sup>e</sup>		15		16		15		16	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 24.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of chlorimuron and glyphosate for common lambsquarters and giant foxtail.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CHEAL				SETFA			
		FWR <sup>b</sup>		HR		FWR		HR	
Chlorimuron	Glyphosate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ae/ha	% Reduction							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	15	-	12	-	2	-	1
2.93	0	-	9	-	20	-	7	-	0
4.4	0	-	29	-	18	-	2	-	2
8.8	0	-	22	-	15	-	10	-	3
0	210	-	40	-	33	-	13	-	13
0	280	-	42	-	36	-	32	-	37
0	420	-	65	-	73	-	69	-	45
0	840	-	83	-	83	-	93	-	69
2.2	210	50	50	43	62 (+)	14	18	14	16
2.93	210	45	49	41	48	19	26	13	14
4.4	210	54	65	44	59	14	34 (+)	15	19
8.8	210	49	60	39	63 (+)	21	22	15	8
2.2	280	52	55	44	60	34	37	38	36
2.93	280	47	49	43	51	37	29	37	24
4.4	280	56	57	45	60	33	44	38	31
8.8	280	53	76 (+)	42	76 (+)	38	35	38	32
2.2	420	66	85 (+)	74	78	70	81	46	48
2.93	420	67	80	75	78	70	81	45	51
4.4	420	73	84	75	82	69	89 (+)	46	59
8.8	420	69	76	76	74	71	76	46	50
2.2	840	84	91	84	85	93	96	70	74
2.93	840	84	91	84	84	94	96	69	69
4.4	840	87	90	84	83	93	97	69	70
8.8	840	85	90	85	84	94	97	69	74
LSD (0.05) <sup>e</sup>		18		17		18		17	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CHEAL, common lambsquarters; SETFA, giant foxtail; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.



**Table 25.** Percent control compared to the untreated for combinations of glyphosate and chlorimuron on giant foxtail 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Giant foxtail							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Chlorimuron g ai/ha	Glyphosate g ae/ha	-----%							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	0	-	0	-	4	-	15
2.93	0	-	7	-	4	-	0	-	21
4.4	0	-	7	-	4	-	3	-	20
8.8	0	-	7	-	8	-	6	-	29
0	210	-	16	-	66	-	71	-	40
0	280	-	19	-	80	-	88	-	50
0	420	-	18	-	93	-	87	-	68
0	840	-	41	-	98	-	85	-	91
2.2	210	16	18	66	69	72	84 (+)	49	44
2.93	210	21	20	68	88 (+)	71	89 (+)	53	45
4.4	210	21	9 (-)	68	89 (+)	72	85 (+)	52	45
8.8	210	21	10 (-)	69	59 (-)	73	86 (+)	57	51
2.2	280	19	13 (-)	80	86	89	89	57	55
2.93	280	24	9 (-)	81	91 (+)	88	93	61	55
4.4	280	24	13 (-)	81	89	88	91	60	55
8.8	280	24	11 (-)	81	92 (+)	89	92	64	55
2.2	420	18	11 (-)	93	97	87	91	72	70
2.93	420	24	23	93	95	87	89	75	69
4.4	420	24	18 (-)	93	98	87	88	75	77
8.8	420	24	19 (-)	94	96	88	89	77	72
2.2	840	41	29 (-)	98	98	86	89	92	89
2.93	840	45	33 (-)	98	98	85	87	93	93
4.4	840	45	31 (-)	98	99	85	91	93	86
8.8	840	45	41	98	100	86	91	93	93
LSD (0.05) <sup>e</sup>		5		9		9		4	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 26.** Percent control compared to the untreated for combinations of chlorimuron and glufosinate on Canada thistle 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Canada thistle							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	28	-	50	-	59	-	73
2.93	0	-	30	-	46	-	54	-	64
4.4	0	-	33	-	60	-	64	-	71
8.8	0	-	38	-	60	-	64	-	71
0	118	-	49	-	39	-	41	-	46
0	157	-	73	-	43	-	53	-	63
0	235	-	74	-	60	-	56	-	58
0	470	-	90	-	91	-	83	-	81
2.2	118	63	81 (+)	69	83 (+)	76	79	86	79
2.93	118	64	83 (+)	67	86 (+)	73	81	81	84
4.4	118	65	91 (+)	75	95 (+)	79	90 (+)	85	91
8.8	118	68	89 (+)	76	91 (+)	79	94 (+)	84	100
2.2	157	80	79	74	79	82	78	90	81
2.93	157	81	85	70	93 (+)	78	87	87	93
4.4	157	81	85	79	93	84	89	90	91
8.8	157	83	90	77	98 (+)	83	94	89	99
2.2	235	81	76	80	68	81	66 (-)	88	69
2.93	235	82	83	78	81	80	78	84	80
4.4	235	82	90	84	95	84	93	87	96
8.8	235	83	91	84	95	84	94	88	96
2.2	470	93	81 (-)	97	81 (-)	94	79 (-)	95	81
2.93	470	93	73 (-)	96	73 (-)	92	70 (-)	94	71
4.4	470	93	89	98	89	94	89	95	90
8.8	470	94	88	96	93	94	93	94	95
LSD (0.05) <sup>e</sup>		9		13		11		11	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 27.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of chlorimuron and glufosinate for canada thistle and velvetleaf.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CIRAR <sup>b</sup>				ABUTH			
		FWR		HR		FWR		HR	
Chlorimuron	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	% Reduction							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	63	-	76	-	59	-	55
2.93	0	-	54	-	79	-	54	-	54
4.4	0	-	65	-	76	-	68	-	68
8.8	0	-	71	-	82	-	74	-	72
0	118	-	50	-	42	-	29	-	30
0	157	-	50	-	38	-	47	-	41
0	235	-	51	-	56	-	51	-	46
0	470	-	79	-	75	-	63	-	67
2.2	118	84	73	87	83	74	56 (-)	76	65
2.93	118	76	79	88	81	72	74	75	76
4.4	118	85	88	87	88	79	81	81	81
8.8	118	84	98	89	94	84	80	83	77
2.2	157	85	66	86	86	89	72 (-)	85	74 (-)
2.93	157	76	87	87	87	89	82	85	78
4.4	157	85	93	88	83	92	85	88	81
8.8	157	84	97	89	89	93	92	88	85
2.2	235	84	73	88	79	87	83	84	78
2.93	235	78	68	90	68 (-)	86	87	84	82
4.4	235	84	91	88	90	90	82	88	82
8.8	235	85	96	92	86	91	89	88	83
2.2	470	94	84	95	82	90	87	89	82
2.93	470	92	67 (-)	95	80	90	91	89	85
4.4	470	93	94	95	84	93	92	92	84
8.8	470	94	86	95	86	94	90	92	84
LSD (0.05) <sup>e</sup>		20		16		14		9	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CIRAR, canada thistle; ABUTH, velvetleaf; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 28.** Percent control compared to the untreated for combinations of chlorimuron and glufosinate on velvetleaf 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Velvetleaf							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	53	-	4	-	28	-	48
2.93	0	-	56	-	8	-	31	-	51
4.4	0	-	59	-	23	-	40	-	64
8.8	0	-	66	-	60	-	52	-	69
0	118	-	38	-	93	-	31	-	16
0	157	-	60	-	95	-	59	-	37
0	235	-	68	-	94	-	46	-	35
0	470	-	88	-	93	-	66	-	54
2.2	118	78	75	93	79 (-)	49	52	62	53
2.93	118	78	79	93	85	50	68 (+)	66	69
4.4	118	79	88 (+)	94	86	56	77 (+)	73	75
8.8	118	83	86	97	88	65	78 (+)	79	82
2.2	157	93	89	95	79	73	64	82	60
2.93	157	93	84 (-)	95	90	74	76	86	76
4.4	157	93	89	96	85 (-)	79	79	90	73
8.8	157	94	89	98	93	84	89	90	89
2.2	235	92	83 (-)	94	91	61	81 (+)	78	84
2.93	235	93	88	94	88 (-)	63	85 (+)	81	84
4.4	235	93	89	95	80	68	84 (+)	86	80
8.8	235	94	88	98	84	74	86 (+)	87	83
2.2	470	97	84 (-)	93	86	75	89 (+)	80	90
2.93	470	97	89	93	90	76	89 (+)	83	91
4.4	470	97	86 (-)	94	91	79	89	87	88
8.8	470	98	88 (-)	97	90 (-)	82	86	89	83
LSD (0.05) <sup>e</sup>		8		6		10		12	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 29.** Percent control compared to the untreated for combinations of chlorimuron and glufosinate on common lambsquarters 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Common lambsquarters							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	36	-	28	-	22	-	8
2.93	0	-	37	-	26	-	19	-	11
4.4	0	-	35	-	28	-	18	-	12
8.8	0	-	38	-	30	-	22	-	21
0	118	-	15	-	41	-	39	-	21
0	157	-	61	-	66	-	69	-	61
0	235	-	65	-	69	-	74	-	64
0	470	-	73	-	61	-	61	-	68
2.2	118	46	66 (+)	58	39 (-)	52	33 (-)	27	33
2.93	118	46	71 (+)	58	47 (-)	50	47 (-)	29	48
4.4	118	45	71 (+)	59	44 (-)	50	39 (-)	31	39
8.8	118	47	66 (+)	59	44 (-)	52	43 (-)	37	43
2.2	157	75	74	74	54 (-)	76	54 (-)	67	59
2.93	157	76	74	73	61 (-)	75	64 (-)	66	66
4.4	157	75	76	73	63 (-)	74	64 (-)	68	68
8.8	157	76	81 (+)	75	74	75	73	69	70
2.2	235	78	81	77	85 (+)	80	79	70	76
2.93	235	78	81	76	87 (+)	80	74	69	74
4.4	235	77	89 (+)	77	86 (+)	79	76	71	75
8.8	235	78	84 (+)	78	68 (-)	80	63 (-)	72	61
2.2	470	82	88 (+)	70	82 (+)	70	85 (+)	72	73
2.93	470	83	88 (+)	68	86 (+)	69	86 (+)	72	81
4.4	470	82	15 (-)	69	63	68	74	73	59
8.8	470	83	25 (-)	72	63 (-)	69	70	74	58
LSD (0.05) <sup>e</sup>		5		7		9		12	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 30.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of chlorimuron and glufosinate for common lambsquarters and giant foxtail.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CHEAL <sup>b</sup>				SETFA			
		FWR		HR		FWR		HR	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	% Reduction							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	11	-	23	-	10	-	5
2.93	0	-	24	-	31	-	20	-	12
4.4	0	-	22	-	35	-	14	-	5
8.8	0	-	12	-	46	-	26	-	11
0	118	-	45	-	26	-	84	-	66
0	157	-	70	-	57	-	99	-	98
0	235	-	79	-	60	-	99	-	98
0	470	-	69	-	63	-	99	-	98
2.2	118	51	59	47	48	85	95 (+)	66	88
2.93	118	57	59	47	59	87	99 (+)	70	96
4.4	118	55	52	54	65	87	99 (+)	67	97
8.8	118	52	55	60	61	88	98 (+)	70	91
2.2	157	72	76	73	62	99	99	98	97
2.93	157	76	75	75	73	99	99	98	98
4.4	157	76	69	79	73	99	99	98	98
8.8	157	75	84	81	77	99	99	98	98
2.2	235	81	82	73	76	99	99	98	98
2.93	235	82	71	76	74	99	99	98	98
4.4	235	82	79	80	72	99	99	98	98
8.8	235	82	66	82	73	99	99	98	98
2.2	470	71	77	76	75	99	99	98	98
2.93	470	74	86	76	84	99	99	98	98
4.4	470	74	98 (+)	81	88	99	99	98	98
8.8	470	73	98 (+)	83	89	99	99	98	98
LSD (0.05) <sup>e</sup>		16		19		6		7	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CHEAL, common lambsquarters; SETFA, giant foxtail; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 31.** Percent control compared to the untreated for combinations of chlorimuron and glufosinate on giant foxtail 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Giant foxtail							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
2.2	0	-	0	-	5	-	5	-	0
2.93	0	-	3	-	5	-	5	-	0
4.4	0	-	3	-	5	-	5	-	0
8.8	0	-	2	-	18	-	14	-	2
0	118	-	76	-	68	-	74	-	54
0	157	-	78	-	76	-	97	-	100
0	235	-	77	-	78	-	94	-	100
0	470	-	80	-	80	-	96	-	100
2.2	118	76	77	69	79 (+)	75	93 (+)	54	83
2.93	118	77	76	69	88 (+)	75	94 (+)	54	99
4.4	118	77	70 (-)	69	93 (+)	75	96 (+)	54	99
8.8	118	77	81	73	81 (+)	77	88 (+)	55	97
2.2	157	78	79	77	86 (+)	97	99	100	100
2.93	157	78	82	77	83	97	98	100	100
4.4	157	78	88 (+)	77	86 (+)	97	99	100	100
8.8	157	78	88 (+)	81	91 (+)	97	98	100	100
2.2	235	77	87 (+)	78	85	95	97	100	100
2.93	235	77	90 (+)	78	79	95	99	100	100
4.4	235	77	93 (+)	79	89 (+)	95	99	100	100
8.8	235	77	88 (+)	82	88	95	98	100	99
2.2	470	80	88 (+)	81	84	96	99	100	100
2.93	470	80	89 (+)	81	86	96	98	100	100
4.4	470	80	90 (+)	81	86	96	99	100	100
8.8	470	80	86 (+)	84	81	96	100	100	100
LSD (0.05) <sup>e</sup>		5		7		7		8	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing chlorimuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 32.** Percent control compared to the untreated for combinations of thifensulfuron and glufosinate on Canada thistle 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Canada thistle							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Thifensulfuron	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
1.125	0	-	4	-	5	-	8	-	10
1.5	0	-	3	-	0	-	5	-	10
2.25	0	-	0	-	0	-	5	-	10
4.5	0	-	6	-	5	-	8	-	10
0	118	-	55	-	44	-	44	-	45
0	157	-	80	-	80	-	66	-	53
0	235	-	79	-	76	-	59	-	41
0	470	-	91	-	100	-	93	-	85
1.125	118	57	74 (+)	46	71 (+)	48	74 (+)	51	76
1.5	118	56	65	44	78 (+)	47	46	51	15
2.25	118	55	46	44	64 (+)	47	58 (+)	51	53
4.5	118	58	59	47	68 (+)	49	48	51	29
1.125	157	81	61 (-)	81	76	69	58 (-)	57	40
1.5	157	81	70 (-)	80	74	68	55 (-)	57	36
2.25	157	80	75	80	69	68	53 (-)	57	36
4.5	157	81	75	81	69	69	63	57	58
1.125	235	80	85	78	86	62	79 (+)	47	73
1.5	235	79	86	76	91 (+)	61	61	47	30
2.25	235	79	84	76	84	61	64	47	44
4.5	235	80	86	77	93 (+)	62	76	47	60
1.125	470	92	96	100	100	93	87	87	74
1.5	470	91	94	100	100	93	77 (-)	87	54
2.25	470	91	94	100	98	93	76 (-)	87	54
4.5	470	92	99	100	98	93	85 (-)	87	73
LSD (0.05) <sup>e</sup>		9		13		8		9	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.



**Table 33.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of thifensulfuron and glufosinate for canada thistle and velvetleaf.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CIRAR <sup>b</sup>				ABUTH			
		FWR		HR		FWR		HR	
Thifensulfuron	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	% Reduction							
0	0	-	0	-	0	-	0	-	0
1.125	0	-	7	-	8	-	20	-	5
1.5	0	-	3	-	17	-	12	-	2
2.25	0	-	0	-	12	-	17	-	1
4.5	0	-	12	-	27	-	19	-	6
0	118	-	28	-	40	-	26	-	21
0	157	-	64	-	73	-	50	-	47
0	235	-	59	-	64	-	71	-	58
0	470	-	95	-	88	-	91	-	75
1.125	118	33	40	45	34	37	75	25	49 (+)
1.5	118	30	48	51	56	31	41	22	39 (+)
2.25	118	28	26	48	34	36	43	22	41 (+)
4.5	118	36	37	57	45	35	56	25	45 (+)
1.125	157	66	39 (-)	75	53 (-)	56	65	50	50
1.5	157	65	31 (-)	77	40 (-)	53	34 (-)	48	39
2.25	157	64	20 (-)	76	55 (-)	55	69	47	59
4.5	157	69	40 (-)	80	41 (-)	57	72	51	59
1.125	235	59	71	66	73	73	86	59	65
1.5	235	59	87 (+)	68	74	73	84	58	66
2.25	235	59	81	68	73	75	71	58	63
4.5	235	63	85	72	81	75	84	60	67
1.125	470	96	96	89	88	94	93	77	79
1.5	470	95	97	90	85	92	89	75	79
2.25	470	95	97	90	86	92	90	75	78
4.5	470	96	98	91	89	93	90	77	77
LSD (0.05) <sup>e</sup>		24		18		20		12	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CIRAR, canada thistle; ABUTH, velvetleaf; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing thifensulfuron also included crop oil concentrate 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 34.** Percent control compared to the untreated for combinations of thifensulfuron and glufosinate on velvetleaf 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Velvetleaf							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
Thifensulfuron	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
1.125	0	-	3	-	1	-	0	-	0
1.5	0	-	8	-	9	-	6	-	1
2.25	0	-	16	-	16	-	9	-	3
4.5	0	-	17	-	17	-	6	-	13
0	118	-	37	-	38	-	20	-	11
0	157	-	43	-	39	-	30	-	20
0	235	-	62	-	61	-	42	-	37
0	470	-	73	-	71	-	64	-	72
1.125	118	38	61 (+)	38	58 (+)	20	45 (+)	11	41
1.5	118	42	48	43	41	24	32	12	33
2.25	118	46	61 (+)	47	48	26	36	14	33
4.5	118	47	61 (+)	48	46	25	39 (+)	22	42
1.125	157	44	64 (+)	39	53 (+)	30	33	20	49
1.5	157	47	47	44	44	34	33	21	36
2.25	157	51	66 (+)	49	65 (+)	36	41	22	54
4.5	157	52	68 (+)	49	68 (+)	34	41	30	50
1.125	235	63	64	61	60	42	49	37	60
1.5	235	65	61	65	62	46	53	38	58
2.25	235	67	58 (-)	67	53 (-)	47	44	39	51
4.5	235	68	62	66	67	46	71 (+)	45	59
1.125	470	73	70	71	71	64	72	72	79
1.5	470	75	78	74	76	69	61	72	79
2.25	470	77	77	75	76	70	68	73	83
4.5	470	77	83	74	78	66	71	76	79
LSD (0.05) <sup>e</sup>		7		8		10		10	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 35.** Percent control compared to the untreated for combinations of thifensulfuron and glufosinate on common lambsquarters 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Common lambsquarters							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Thifensulfuron	Glufosinate	-----%-----							
g ai/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
1.125	0	-	24	-	23	-	30	-	49
1.5	0	-	26	-	31	-	42	-	54
2.25	0	-	34	-	41	-	50	-	67
4.5	0	-	35	-	48	-	44	-	58
0	118	-	18	-	19	-	22	-	29
0	157	-	29	-	26	-	25	-	30
0	235	-	41	-	52	-	51	-	50
0	470	-	61	-	61	-	64	-	56
1.125	118	38	47	37	58 (+)	45	61 (+)	64	66
1.5	118	40	64 (+)	43	70 (+)	55	70 (+)	68	73
2.25	118	47	61 (+)	52	70 (+)	61	71 (+)	77	71
4.5	118	47	72 (+)	57	76 (+)	55	74 (+)	67	83
1.125	157	47	69 (+)	43	75 (+)	49	74 (+)	67	75
1.5	157	49	74 (+)	49	73 (+)	57	68 (+)	70	70
2.25	157	54	69 (+)	56	74 (+)	63	70	78	71
4.5	157	54	69 (+)	62	74 (+)	57	73 (+)	68	71
1.125	235	57	72 (+)	64	71 (+)	67	73	77	71
1.5	235	58	78 (+)	67	80 (+)	72	74	79	77
2.25	235	63	78 (+)	72	76	76	74	84	76
4.5	235	62	79 (+)	75	82	72	83 (+)	76	85
1.125	470	71	76	69	78 (+)	74	68	78	71
1.5	470	72	82 (+)	73	82 (+)	78	78	81	80
2.25	470	74	83	77	82	82	77	86	81
4.5	470	74	85 (+)	79	87 (+)	79	88	80	94
LSD (0.05) <sup>e</sup>		9		7		9		12	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 36.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of thifensulfuron and glufosinate for common lambsquarters and giant foxtail.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CHEAL <sup>b</sup>				SETFA			
		FWR		HR		FWR		HR	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	% Reduction							
0	0	-	0	-	0	-	0	-	0
1.125	0	-	41	-	38	-	4	-	0
1.5	0	-	22	-	35	-	4	-	0
2.25	0	-	36	-	50	-	9	-	1
4.5	0	-	26	-	43	-	2	-	1
0	118	-	38	-	18	-	36	-	22
0	157	-	35	-	20	-	85	-	74
0	235	-	45	-	38	-	94	-	88
0	470	-	50	-	44	-	96	-	97
1.125	118	66	59	48	58	38	64 (+)	23	53 (+)
1.5	118	54	63	47	67 (+)	37	55 (+)	22	49 (+)
2.25	118	59	57	60	65	40	53	23	45 (+)
4.5	118	55	70	51	75 (+)	36	65 (+)	23	39 (+)
1.125	157	62	67	49	65 (+)	86	77	74	62
1.5	157	47	53	46	62 (+)	86	82	74	67
2.25	157	59	63	59	63	87	81	74	71
4.5	157	48	64	53	69 (+)	86	84	74	71
1.125	235	72	59	62	60	94	79 (-)	88	82
1.5	235	60	69	59	68	94	92	88	89
2.25	235	64	71	69	71	94	94	88	88
4.5	235	61	72	62	77	94	88	88	79
1.125	470	67	53	63	60	97	94	97	98
1.5	470	59	72	63	66	97	96	97	97
2.25	470	68	67	70	73	97	96	97	96
4.5	470	56	90 (+)	68	85 (+)	96	97	97	97
LSD (0.05) <sup>e</sup>		25		15		14		16	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CHEAL, common lambsquarters; SETFA, giant foxtail; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing thifensulfuron also included crop oil concentrate 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 37.** Percent control compared to the untreated for combinations of thifensulfuron and glufosinate on giant foxtail 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Giant foxtail							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Thifensulfuron	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ai/ha	g ai/ha	-----%-----							
0	0	-	0	-	0	-	0	-	0
1.125	0	-	7	-	1	-	3	-	0
1.5	0	-	12	-	1	-	4	-	0
2.25	0	-	22	-	6	-	1	-	0
4.5	0	-	18	-	5	-	0	-	0
0	118	-	28	-	69	-	43	-	29
0	157	-	40	-	81	-	88	-	61
0	235	-	57	-	82	-	91	-	77
0	470	-	73	-	84	-	91	-	91
1.125	118	34	44	69	82 (+)	44	86 (+)	29	61
1.5	118	38	53 (+)	69	83 (+)	45	84 (+)	29	58
2.25	118	44	42	71	76	43	74 (+)	29	53
4.5	118	40	56 (+)	70	81 (+)	43	78 (+)	29	54
1.125	157	45	60 (+)	82	84	89	85	61	64
1.5	157	48	49	82	85	88	86	61	68
2.25	157	54	61	82	83	88	84	61	58
4.5	157	50	61 (+)	82	83	88	83	61	72
1.125	235	60	71 (+)	82	73 (-)	91	86	77	71
1.5	235	63	68	82	84	91	85	77	70
2.25	235	67	66	83	88	91	87	77	79
4.5	235	64	74	83	86	91	86	77	71
1.125	470	75	74	85	88	91	84	91	91
1.5	470	77	79	85	87	91	86	91	95
2.25	470	79	83	85	88	91	86	91	97
4.5	470	77	81	85	88	91	88	91	94
LSD (0.05) <sup>e</sup>		11		7		8		9	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glufosinate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 38.** Percent control compared to the untreated for combinations of glyphosate and thifensulfuron on Canada thistle 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Canada thistle							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Glyphosate	Thifensulfuron	-----%							
g ae/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
210	0	-	69	-	81	-	79	-	83
280	0	-	89	-	95	-	98	-	99
420	0	-	81	-	98	-	98	-	99
840	0	-	90	-	100	-	99	-	94
0	1.125	-	35	-	7	-	4	-	9
0	1.5	-	44	-	9	-	14	-	13
0	2.25	-	45	-	13	-	13	-	11
0	4.5	-	45	-	26	-	29	-	6
210	1.125	76	75	82	87	79	78	83	84
210	1.5	77	73	83	83	80	73	83	78
210	2.25	77	71	83	85	80	80	83	78
210	4.5	77	74	83	84	83	83	83	84
280	1.125	89	78 (-)	95	90	98	87	99	86
280	1.5	89	79 (-)	95	92	98	88	99	91
280	2.25	89	75 (-)	95	93	98	89	99	94
280	4.5	89	79 (-)	95	98	98	94	99	100
420	1.125	89	83	98	97	98	94	99	93
420	1.5	91	87	98	98	98	94	99	96
420	2.25	90	86	98	96	98	93	99	94
420	4.5	91	79 (-)	98	94	98	96	99	98
840	1.125	93	89	100	99	99	99	98	100
840	1.5	94	90	100	99	99	100	100	100
840	2.25	95	88	100	98	99	99	99	100
840	4.5	95	85 (-)	100	93	99	93	97	92
LSD (0.05) <sup>e</sup>		8		8		14		14	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 39.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of glyphosate and thifensulfuron on Canada thistle and velvetleaf.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CIRAR <sup>b</sup>				ABUTH			
		FWR		HR		FWR		HR	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Glyphosate	Thifensulfuron	% Reduction							
g ae/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
210	0	-	81	-	76	-	74	-	76
280	0	-	95	-	80	-	87	-	83
420	0	-	95	-	83	-	95	-	93
840	0	-	96	-	83	-	97	-	91
0	1.125	-	0	-	1	-	13	-	9
0	1.5	-	2	-	4	-	27	-	12
0	2.25	-	6	-	4	-	20	-	15
0	4.5	-	7	-	7	-	31	-	23
210	1.125	81	83	76	79	75	80	78	78
210	1.5	81	71 (-)	76	75	78	73	79	76
210	2.25	82	80	77	73	76	73	79	75
210	4.5	82	87	77	74	77	88	81	81
280	1.125	95	91	80	78	88	89	84	85
280	1.5	95	89	80	77	90	90	85	80
280	2.25	95	93	80	82	90	90	85	81
280	4.5	95	95	81	81	90	88	86	80
420	1.125	95	93	83	80	96	93	94	90
420	1.5	95	95	83	78	96	94	94	92
420	2.25	96	95	83	82	96	89	94	92
420	4.5	96	92	84	81	96	96	95	94
840	1.125	96	95	83	83	97	95	91	93
840	1.5	96	96	84	85	97	94	92	93
840	2.25	96	96	84	83	97	94	92	93
840	4.5	96	93	84	81	97	95	93	93
LSD (0.05) <sup>e</sup>		8		6		12		8	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CIRAR, Canada thistle; ABUTH, velvetleaf; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 40.** Percent control compared to the untreated for combinations of glyphosate and thifensulfuron on velvetleaf 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Velvetleaf							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Glyphosate	Thifensulfuron	-----%-----							
g ae/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
210	0	-	39	-	53	-	60	-	73
280	0	-	44	-	74	-	80	-	88
420	0	-	44	-	83	-	97	-	98
840	0	-	45	-	96	-	87	-	98
0	1.125	-	19	-	5	-	5	-	9
0	1.5	-	10	-	5	-	5	-	13
0	2.25	-	20	-	18	-	9	-	17
0	4.5	-	23	-	26	-	24	-	25
210	1.125	51	50	56	65	60	67	74	77
210	1.5	45	47	56	61	60	69	76	71
210	2.25	51	38 (-)	62	68	63	71	76	73
210	4.5	53	54	65	76	70	83	77	79
280	1.125	54	45	75	75	80	79	89	89
280	1.5	49	46	76	77	80	84	90	84
280	2.25	55	54	78	68	82	83	89	84
280	4.5	56	54	80	71	85	82	90	89
420	1.125	55	46 (-)	84	89	97	94	98	95
420	1.5	50	56	84	94	97	98	98	97
420	2.25	55	50	86	91	97	91	98	92
420	4.5	57	58	87	93	98	98	98	96
840	1.125	56	51	96	96	87	99	98	100
840	1.5	51	55	96	97	87	99	98	99
840	2.25	56	50	97	94	88	97	98	98
840	4.5	57	59	97	96	90	99	98	99
LSD (0.05) <sup>e</sup>		7		13		14		13	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.



**Table 41.** Percent control compared to the untreated for combinations of glyphosate and thifensulfuron on common lambsquarters 7, 14, 21 and 28 days after treatment.<sup>a</sup>

		Visual Control - Common Lambsquarters							
Herbicide Rate <sup>c,d</sup>		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
Glyphosate	Thifensulfuron	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
g ae/ha	g ai/ha	-----%							
0	0	-	0	-	0	-	0	-	0
210	0	-	10	-	9	-	21	-	29
280	0	-	13	-	18	-	36	-	34
420	0	-	21	-	38	-	39	-	54
840	0	-	48	-	64	-	63	-	49
0	1.125	-	0	-	6	-	0	-	48
0	1.5	-	0	-	8	-	0	-	33
0	2.25	-	1	-	9	-	3	-	29
0	4.5	-	3	-	11	-	5	-	41
210	1.125	9	11	15	23	21	15	57	62
210	1.5	9	29 (+)	16	26	21	30 (+)	50	58
210	2.25	9	46 (+)	18	48 (+)	24	58 (+)	49	58
210	4.5	18	35 (+)	19	51 (+)	26	65 (+)	60	69
280	1.125	13	9	23	27	36	41	63	66
280	1.5	13	13	24	33	36	43	55	59
280	2.25	13	15	26	33	38	50 (+)	53	61
280	4.5	21	55 (+)	27	47 (+)	40	64 (+)	62	61
420	1.125	20	68 (+)	41	67 (+)	39	64 (+)	70	51
420	1.5	20	44 (+)	42	54	39	64 (+)	67	54
420	2.25	20	43 (+)	43	54	40	60 (+)	66	58
420	4.5	28	26	44	47	42	55 (+)	75	64
840	1.125	43	58 (+)	64	71	63	79 (+)	75	77
840	1.5	43	61 (+)	64	77	63	82 (+)	66	79
840	2.25	43	59 (+)	66	66	63	75 (+)	64	79
840	4.5	49	78 (+)	67	88 (+)	64	85 (+)	69	85
LSD (0.05) <sup>e</sup>		15		15		11		12	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 42.** Fresh weight and height reduction compared to the untreated control 28 days after treatment for combinations of glyphosate and thifensulfuron on common lambsquarters and giant foxtail.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		CHEAL <sup>b</sup>				SETFA			
		FWR		HR		FWR		HR	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Glyphosate	Thifensulfuron	% Reduction							
g ae/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
210	0	-	10	-	46	-	95	-	94
280	0	-	30	-	54	-	96	-	95
420	0	-	71	-	88	-	96	-	97
840	0	-	83	-	86	-	97	-	96
0	1.125	-	14	-	23	-	5	-	8
0	1.5	-	32	-	37	-	19	-	6
0	2.25	-	38	-	28	-	4	-	11
0	4.5	-	31	-	35	-	5	-	7
210	1.125	23	78 (+)	59	87 (+)	96	95	94	91
210	1.5	36	65 (+)	62	87 (+)	96	86 (-)	94	86 (-)
210	2.25	40	81 (+)	59	86 (+)	95	95	95	87 (-)
210	4.5	36	88 (+)	62	85 (+)	95	96	94	94
280	1.125	33	65 (+)	61	90 (+)	96	97	95	97
280	1.5	43	90 (+)	70	86 (+)	96	95	95	97
280	2.25	45	76 (+)	64	91 (+)	96	96	95	97
280	4.5	41	93 (+)	67	95 (+)	96	95	95	97
420	1.125	71	81	91	87	96	96	97	97
420	1.5	72	68	91	84	97	97	97	96
420	2.25	72	82	90	81	96	97	97	97
420	4.5	71	91	91	90	96	96	97	97
840	1.125	83	95	89	91	97	96	97	97
840	1.5	85	96	90	90	97	98	97	97
840	2.25	86	92	89	89	97	97	97	97
840	4.5	85	90	89	90	97	96	97	97
LSD (0.05) <sup>e</sup>		21		13		6		4	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: CHEAL, common lambsquarters; SETFA, giant foxtail; FWR, fresh weight reduction; HR, height reduction; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>d</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 43.** Percent control compared to the untreated for combinations of glyphosate and thifensulfuron on giant foxtail 7, 14, 21 and 28 days after treatment.<sup>a</sup>

Herbicide Rate <sup>c,d</sup>		Visual Control - Giant foxtail							
		7 DAT <sup>b</sup>		14 DAT		21 DAT		28 DAT	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Glyphosate	Thifensulfuron	-----%							
g ae/ha	g ai/ha								
0	0	-	0	-	0	-	0	-	0
210	0	-	33	-	93	-	99	-	89
280	0	-	33	-	96	-	100	-	88
420	0	-	41	-	100	-	100	-	78
840	0	-	39	-	100	-	78	-	100
0	1.125	-	0	-	8	-	5	-	18
0	1.5	-	5	-	9	-	5	-	16
0	2.25	-	0	-	9	-	14	-	16
0	4.5	-	0	-	9	-	13	-	17
210	1.125	33	35	93	88	99	99	100	88
210	1.5	34	41	93	98	99	100	100	100
210	2.25	33	36	93	88	99	99	100	98
210	4.5	33	39	93	96	99	100	100	100
280	1.125	33	58 (+)	97	100	100	100	100	100
280	1.5	34	43	97	98	100	100	100	100
280	2.25	33	36	97	94	100	100	100	100
280	4.5	33	54 (+)	97	96	100	100	100	100
420	1.125	41	46	100	76 (-)	100	100	90	100
420	1.5	42	51	100	98	100	100	90	99
420	2.25	41	45	100	100	100	100	90	100
420	4.5	41	51	100	100	100	100	90	100
840	1.125	40	59 (+)	100	100	79	100 (+)	100	100
840	1.5	40	60 (+)	100	99	79	100 (+)	100	100
840	2.25	39	70 (+)	100	100	80	100 (+)	100	100
840	4.5	39	70 (+)	100	100	80	100 (+)	100	100
LSD (0.05) <sup>e</sup>		7		13		14		13	

<sup>a</sup> + and - denote a synergistic and antagonistic interaction for the given combinations according to the LSD comparison of the observed and expected values calculated using Colby's method. Observed values without a + or - are additive.

<sup>b</sup> Abbreviations: DAT, days after treatment; Exp., expected; Obs., observed.

<sup>c</sup> Treatments containing glyphosate also included ammonium sulfate at 2% v/v.

<sup>d</sup> Treatments containing thifensulfuron also included crop oil concentrate at 1% v/v.

<sup>e</sup> LSD values may be used to compare values.

**Table 44. Fv/Fm values of Canada thistle in fluorescence studies<sup>a</sup>**

Herbicide Applied	2 HAT <sup>a</sup>	4 HAT	6 HAT	8 HAT	24 HAT	48 HAT	72 HAT
CONTROL	0.81 ab	0.81 a	0.81 a	0.81 a	0.78 a	0.64 a	0.58 abc
0.25X GLY	0.82 ab	0.81 a	0.80 a	0.78 ab	0.69 abcde	0.44 b	0.48 bcd
0.5X GLY	0.82 a	0.81 a	0.78 ab	0.75 ab	0.65 cde	0.34 bcde	0.42 cdef
1X GLY	0.81 ab	0.80 a	0.79 ab	0.76 ab	0.67 bcde	0.40 bc	0.26 fghij
0.25X GLU	0.74 fgh	0.63 de	0.48 de	0.43 ef	0.32 fg	0.23 defg	0.21 ghijk
0.5X GLU	0.71 h	0.60 e	0.49 cde	0.45 def	0.29 fg	0.19 efg	0.10 jklm
1X GLU	0.72 gh	0.61 de	0.49 cde	0.45 def	0.35 fg	0.08 g	0.13 iklm
0.25X CHL	0.82 ab	0.81 a	0.79 ab	0.78 ab	0.76 ab	0.65 a	0.61 ab
0.5X CHL	0.81 ab	0.81 a	0.80 a	0.79 a	0.76 ab	0.65 a	0.68 a
1X CHL	0.81 abc	0.81 a	0.72 b	0.79 a	0.74 abc	0.67 a	0.57 abc
0.25X GLU + 1X GLY	0.79 abcd	0.67 cde	0.49 cde	0.47 cde	0.32 fg	0.14 g	0.03 m
0.5X GLU + 0.5X GLY	0.77 cdef	0.63 de	0.52 cde	0.50 cd	0.28 g	0.15 fg	0.06 lm
1X GLU + 0.25X GLY	0.75 efg	0.61 e	0.47 e	0.41 f	0.27 g	0.16 fg	0.10 jklm
0.25 GLU + 0.25X CHL	0.80 abc	0.62 de	0.56 c	0.51 c	0.35 fg	0.19 efg	0.05 lm
0.25X GLU + 1X CHL	0.79 bcde	0.69 cd	0.56 c	0.51 c	0.38 f	0.21 defg	0.13 iklm
0.5X GLU + 0.5X CHL	0.79 bcde	0.65 cde	0.55 cd	0.50 cd	0.32 fg	0.21 defg	0.10 jklm
1X GLU + 0.25X CHL	0.75 efg	0.65 cde	0.52 cde	0.50 cd	0.31 fg	0.18 fg	0.06 klm
1X GLU + 0.5X CHL	0.76 efg	0.64 de	0.52 cde	0.49 cde	0.29 fg	0.14 fg	0.14 iklm
1X GLU + 1X CHL	0.76 def	0.65 cde	0.56 c	0.51 cd	0.34 fg	0.18 fg	0.24 fghijk
0.25X GLY + 0.25X CHL	0.81 ab	0.80 a	0.76 ab	0.73 b	0.67 bcde	0.46 b	0.47 bcd
0.25X GLY + 1X CHL	0.82 a	0.72 bc	0.80 a	0.79 ab	0.72 abcd	0.47 b	0.46 bcde
0.5X GLY + 0.5X CHL	0.83 a	0.81 a	0.80 ab	0.75 ab	0.68 bcde	0.36 bcd	0.33 defg
1X GLY + 0.25X CHL	0.80 abc	0.79 ab	0.77 ab	0.76 ab	0.63 de	0.34 bcde	0.28 efghi
1X GLY + 0.5X CHL	0.82 a	0.81 a	0.79 ab	0.73 b	0.62 de	0.34 bcde	0.32 defghi
1X GLY + 1X CHL	0.81 ab	0.77 ab	0.74 ab	0.77 ab	0.61 e	0.29 cdef	0.15 hijklm
LSD (0.05) <sup>b</sup>	0.04	0.08	0.08	0.06	0.10	0.15	0.18

<sup>a</sup> Abbreviations: CHL, chlorimuron; HAT, hours after treatment; GLU, glufosinate; GLY, glyphosate

<sup>b</sup> LSD values are computed at the 0.05 significance level and may be used to compare values

**Table 45. Fv/Fm values of common lambsquarters in fluorescence studies<sup>a</sup>**

Herbicide Applied	2 HAT <sup>a</sup>	4 HAT	6 HAT	8 HAT	24 HAT	48 HAT	72 HAT
CONTROL	0.74 abc	0.79 abc	0.72 abcde	0.72 abcd	0.64 ab	0.65 abcd	0.68 a
0.25X GLY	0.60 d	0.62 f	0.63 bcdefg	0.66 abcde	0.54 bc	0.54 abcde	0.64 ab
0.5X GLY	0.66 bcd	0.76 abcd	0.72 abcde	0.72 abcd	0.61 ab	0.62 abcd	0.60 abcd
1X GLY	0.71 abcd	0.77 abcd	0.68 abcde	0.71 abcd	0.61 ab	0.46 bcdef	0.39 cdef
0.25X GLU	0.74 abc	0.77 abcd	0.68 abcde	0.66 abcde	0.38 def	0.24 fghi	0.26 efgh
0.5X GLU	0.69 abcd	0.72 bcde	0.55 gh	0.52 fg	0.23 fgh	0.36 efghi	0.42 bcde
1X GLU	0.69 abcd	0.74 abcd	0.55 fgh	0.50 g	0.24 efgh	0.28 fghi	0.10 gh
0.25X CHL	0.75 abc	0.80 ab	0.72 abcde	0.78 a	0.67 ab	0.65 abcd	0.62 abc
0.5X CHL	0.78 abc	0.80 a	0.76 a	0.74 abc	0.72 a	0.74 a	0.69 a
1X CHL	0.66 bcd	0.72 abcde	0.67 abcdefg	0.74 abc	0.67 ab	0.70 ab	0.73 a
0.25X GLU + 1X GLY	0.69 abcd	0.72 bcde	0.65 abcdefg	0.65 bcdef	0.41 cd	0.32 efghi	0.32 efg
0.5X GLU + 0.5X GLY	0.79 abc	0.78 abc	0.67 abcdef	0.60 defg	0.18 gh	0.17 hi	0.04 h
1X GLU + 0.25X GLY	0.68 abcd	0.71 cde	0.55 fgh	0.50 g	0.09 h	0.17 i	0.18 fgh
0.25 GLU + 0.25X CHL	0.68 abcd	0.66 ef	0.62 cdefgh	0.64 bcdef	0.35 def	0.42 defg	0.20 efgh
0.25X GLU + 1X CHL	0.78 ab	0.78 abc	0.65 abcdefg	0.64 bcdef	0.35 def	0.42 defgh	0.39 def
0.5X GLU + 0.5X CHL	0.73 abc	0.73 abcde	0.61 defgh	0.63 cdefg	0.29 defg	0.31 efghi	0.17 fgh
1X GLU + 0.25X CHL	0.72 abcd	0.77 abcd	0.60 fgh	0.57 efg	0.15 gh	0.22 fghi	0.11 gh
1X GLU + 0.5X CHL	0.69 abcd	0.69 def	0.50 h	0.52 fg	0.10 h	0.19 ghi	0.30 efg
1X GLU + 1X CHL	0.66 cd	0.71 cde	0.61 defgh	0.57 efg	0.39 cde	0.46 bcdef	0.35 ef
0.25X GLY + 0.25X CHL	0.70 abcd	0.80 a	0.74 abc	0.77 ab	0.72 a	0.65 abcd	0.68 a
0.25X GLY + 1X CHL	0.68 abcd	0.75 abcd	0.75 ab	0.75 abc	0.70 ab	0.67 abc	0.74 a
0.5X GLY + 0.5X CHL	0.63 cd	0.77 abcd	0.74 abc	0.74 abc	0.67 ab	0.68 ab	0.67 a
1X GLY + 0.25X CHL	0.71 abcd	0.76 abcd	0.74 ab	0.71 abcd	0.67 ab	0.69 ab	0.70 a
1X GLY + 0.5X CHL	0.67 abcd	0.76 abcd	0.73 abcd	0.75 abc	0.67 ab	0.43 cdefg	0.58 abcd
1X GLY + 1X CHL	0.66 bcd	0.75 abcd	0.74 abc	0.75 abc	0.70 ab	0.46 bcdef	0.60 abcd
LSD (0.05) <sup>b</sup>	0.1185	0.0807	0.1246	0.133	0.1604	0.2474	0.2276

<sup>a</sup> Abbreviations: CHL, chlorimuron; HAT, hours after treatment; GLU, glufosinate; GLY, glyphosate

<sup>b</sup> LSD values are computed at the 0.05 significance level and may be used to compare values

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