WEED CONTROL AND TANK-MIX INTERACTIONS IN SOYBEAN RESISTANT TO DICAMBA, GLYPHOSATE, AND GLUFOSINATE

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ABSTRACT

WEED CONTROL AND TANK-MIX INTERACTIONS IN SOYBEAN RESISTANT TO DICAMBA, GLYPHOSATE, AND GLUFOSINATE

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XtendFlex® soybean is a new trait platform which confers resistance to three herbicide sites of action that include the active ingredients dicamba, glyphosate, and glufosinate. The ability to use these three herbicides in one system has generated new management questions. Field and greenhouse experiments were conducted in 2019 and 2020 to: 1) investigate weed control systems in conventional and no-tillage XtendFlex® soybean, and 2) identify any antagonistic or synergistic responses from herbicide-tank mixtures used in this system. Control of glyphosate-resistant (GR) waterhemp was optimized with PRE flumioxazin followed by (fb) POST glufosinate or dicamba alone or in combination with each other or glyphosate. Two-pass POST systems also controlled GR waterhemp as long as dicamba and/or glufosinate was used in each application. GR horseweed control was exceptional with all herbicide programs evaluated, except glyphosate alone EPOS or POST. Annual grass control was reduced with EPOS and POST glufosinate + dicamba tank-mixtures. In contrast, this combination was often additive or synergistic for both broadleaf and grass weed control in the greenhouse. Several glyphosate + glufosinate combinations were antagonistic, especially with broadleaf weeds. Dicamba + glyphosate was often antagonistic in the greenhouse but was additive or synergistic for GR waterhemp and GR horseweed control in the field. Antagonisms were often observed when all three herbicides were applied together; however, not all antagonisms resulted in poor control. This research provides growers insight into management strategies for various agronomically important weeds in XtendFlex® soybean.

Dedicated to:
my parents, Leo and Lori Constine;
my fiancé, Karlee VanHorn;
and my lifelong mentor in agriculture, James Newton

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CHAPTER I

LITERATURE REVIEW

Soybean

Soybean, taxonomically classified as *Glycine max* (L.) Merr., is one of the fundamental agronomic crops grown in the United States. Soybean-based protein products are a staple for livestock production and human consumption across the globe. The United States is the leading producer of soybean and ranks second in global exports (USDA-ERS 2020a). In 2019, soybean was planted on 30.8 million hectares in the United States, producing 96.8 million metric tons with a cash value of $31.2 billion USD (USDA-ERS 2020b). Ranked 4th in global production, 85% of an annual soybean crop is processed into soybean meal, a high protein animal feed, and vegetable oil; within the United States, soybean is responsible for 90% of oilseed production (USDA-ERS 2020a). When including its derivatives, soybean is the most traded agricultural commodity, accounting for 20 percent of agricultural trade across the world during the 2016/17 marketing year (USDA-ERS 2020a).

Soybean is an economically important crop in the state of Michigan. In 2019, soybean was the second most produced crop in the state, accounting for 37.3% of all planted hectares in Michigan with production valued at $631 million USD (USDA-NASS 2020). The development and commercialization of herbicide-resistant soybean varieties has increased profitability of soybean acres across the nation. However, recent shifts in economically important weed populations towards herbicide-resistant biotypes has many growers concerned for the future of US soybean production.

Weed Control in Soybean

Weed management has continued to be an important component in soybean production. Weedy species compete with soybean for light, nutrients, and water, reducing crop yield and limiting economic returns (Harre and Young 2020; Monks and Oliver 1988; Munger et al. 1987; Patterson 1995; Stoller and Woolley 1985; Zollinger and Kells 1993). Uncontrolled weeds in soybean can reduce yield by up to 79% (Bensch et al. 2003; Coble et al. 1981; Hager et al. 2002; Klingaman and Oliver 1994; Moolani et al. 1964; Shurtleff and Coble 1985; Soltani et al. 2017; Stoller and Woolley 1985).

Soybean yield loss from weed competition is affected by a variety of factors such as, soybean planting date, soybean row width, soybean population, the timing of weed emergence, timing of weed control strategies, species present, population density, and biomass accumulation (Aldrish 1987; Hock et al. 2006; Krausz et al. 2001). To avoid yield loss from competition, weeds should be managed during the critical time of weed removal, which has been defined as the period of time during crop growth in which weeds should be controlled to prevent yield loss (Zimdahl 1988). Many studies have defined this period in soybean to occur between the V2 and V4 growth stages (Ficket et al. 2013; Halford et al. 2001; Mulugeta and Boerboom 2000; Stoller et al. 1987; Van Acker et al. 1993; Zimdahl 1988). Bensch et al. (2003) found that redroot pigweed (*Amaranthus retroflexus* L.), common waterhemp (*A. rudis* J.D. Sauer), and Palmer amaranth (*A. palmeri* S. Watson) reduced soybean yield by 38%, 56%, and 79%, respectively, when 8 seedlings m^{-1} of row were planted at the same time as soybean. Alternatively, soybean yield was not reduced when pigweed species planting was delayed until the cotyledon growth stage (VC). Coble et al. (1981) observed similar yield losses from the interference of common ragweed (*Ambrosia artemisiifolia* L.) in soybean; yield losses of 62%, 17%, and 1% occurred when soybeans were kept weed-free for the first 0, 2, and 4 weeks of the growing season, respectively. Likewise, the interference of common lambsquarters (*Chenopodium album* L.) resulted in a 15% yield loss when emerged with soybean at a density of 16 weeds per 10 m of row (Shurtleff and Coble 1985).

Historically, there are certain weed species that have posed management challenges in soybean produced in Michigan. Annual broadleaves that are challenging to control in soybean include: common lambsquarters, common ragweed, giant ragweed (*Ambrosia trifida* L.), eastern black nightshade (*Solanum ptychanthum* Dunal.), jimsonweed (*Datura stramonium* L.), pigweed species (*Amaranthus retroflexus* L. and *A. powellii* S. Watson), smartweed species (*Persicaria pensylvanica* L. and *P. maculosa* Grey), velvetleaf (*Abutilion theophrasti* Medik.), wild mustard (*Sinapis arvensis* L.), and horseweed (*Erigeron canadensis* L.) (MSU-PSM 2020). Annual grasses can also be troublesome in Michigan soybean systems and require control. Common annual grass species include: foxtail species (*Setaria faberi* Herrm.), *S. pumila* (Pior.) Roem. & Schult., and *S. viridis* (L.) P. Beauv) and fall panicum (*Panicum dichotomiflorum* Michx.) (MSU-PSM 2020). Although there are many weeds that are of concern to Michigan soybean growers, it is common that two to four weed species will dominate the weed population within each field (Wilson and Furrer 1986).

Since their inception in the 1940s, synthetic herbicides have continued to acquire a critical role in crop production systems around the globe, especially in soybean. However, traditional weed management systems in soybean prior to the introduction of glyphosate-resistant (GR) crops relied on the integrated use of cultural, mechanical, and chemical control practices to optimize weed control, economics, and sustainability (Mickelson and Renner 1997; Steckel et al. 1990; Walker and Buchanan 1982). Advancements made in soybean herbicide technology led to an increased reliance on chemical weed control beginning in the 1960s (McWhorter and Barrentine 1966; Wax and McWhorter 1968). In the 1970s, over 30 herbicides were labeled for use in soybean (Wax 1973). Prior to the release of glyphosate-resistant soybean, typical herbicide recommendations for weed control in soybean included the use of soil-applied herbicides preemergence (PRE) to delay weed emergence beyond the critical time of weed removal; followed by the application of a postemergence (POST) herbicide(s) to control remaining weeds (Coble et al. 1981; Gebhardt 1981; Shurtleff and Coble 1985; Steckel et al. 1990; Young 2006). PRE or preplant incorporated (PPI) herbicides used in soybean included alachlor, chlorimuron, imazaquin, imazethapyr, linuron, metolachlor, metribuzin, pendimethalin, and trifluralin (Adcock et al. 1990; Adcock and Banks 1991; Bruce and Kells 1990; Green et al. 1988; Stougaard et al. 1984; Young 2006). Typical POST herbicides included acifluorfen, bentazon, chlorimuron, clethodim, flumiclorac, imazamox, imazaquin, imazethapyr, lactofen, sethoxydim, and thifensulfuron (Defelice et al. 1989; Devlin et al. 1991; Kapusta et al. 1986; Lich et al. 1997; Mayo et al. 1995; Young 2006).

Glyphosate-Resistant Weeds

Following the stable integration of microbial genes responsible for the degradation of glyphosate into important cash crops, glyphosate-resistant (GR) crops were introduced in 1996 and since their introduction, over 185 million hectares of GR crops have been planted in the United States (Dekker and Duke 1995; Gianessi 2005). Prior to the introduction of GR crops, glyphosate use in the United States rose from 0.34 million kg in 1974, to 1.8 million kg in 1990 (Benbrook 2016; Gianessi 2008). Likewise, use of glyphosate continued to rise following the introduction of GR crops, totaling 45 million kg of active ingredient used in 2005 and 125 million kg used in 2014 with 90% being used for agricultural purposes (Benbrook 2016; Gianessi 2008). Glyphosate-resistant crops provided growers with an effective, yet simple solution for broad-spectrum weed control across cropping systems. As a result of glyphosate's effectiveness and simplicity, many growers had abandoned best management practices for avoiding the development of herbicide resistance in weedy species, including mechanical weed control, tank-mixing herbicides with multiple effective sites of action, and crop rotation (Wright et al. 2010). The heavy reliance on a single herbicide, glyphosate, for weed control significantly increased selection pressure for naturally occurring GR traits in weed populations (Owen 2008; Powles 2008; Young 2006). Evans et al. (2016) found that tank mixing multiple effective herbicides can significantly reduce the selection for glyphosate resistance. Applications containing 2.5 modes of action (MOAs) are 83 times less likely to develop glyphosate resistance compared with applications only containing 1.5 MOAs. Likewise, applications containing 3 MOAs were 51 times less likely to develop glyphosate resistance compared with applications containing 2 MOAs. These findings demonstrate the importance of utilizing multiple effective sites of action in delaying the onset of herbicide resistance and explain the ability for weeds to develop widespread herbicide resistance.

The development of herbicide resistance in weeds can be categorized into five main mechanisms: altered-target site, metabolism-based, reduced absorption/translocation, sequestration into vacuoles, and gene amplification (Heap 2014). These mechanisms of resistance can be grouped into two broad categories, target site and non-target site resistance (Dekker and Duke 1995; Delye et al. 2013). Target site resistance to an herbicide is conferred in a plant species in one of two ways. The first way target site resistance is conferred is when structural changes are made to the target site. In this instance, the herbicide molecule is no longer capable of binding to the target site or the affinity for the native substrate becomes greater than that of the herbicide (Delye et al. 2013; Murphy and Tranel 2019). The second way target site resistance is conferred occurs when the target site is overexpressed through gene amplification, which often requires more herbicide to achieve a lethal dose (Gaines et al. 2010; Murphey and Tranel 2019). Target site resistance via altered target site has been confirmed in multiple weed species to nine different target sites or herbicide sites of action, including: acetyl-CoA carboxylase (ACCase) inhibitors (WSSA Group 1), acetolactate synthase (ALS) inhibitors (Group 2), microtubule inhibitors (Group 3), synthetic auxins (Group 4), photosystem II (PSII) inhibitors (Groups 5, 6, and 7), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors (Group 9), glutamine synthetase inhibitors (Group 10), phytoene desaturase inhibitors (Group 12), and protoporphyrinogen oxidase (PPO) inhibitors (Group 14) (Devine and Shukla 2000; Mallory-Smith and Retzinger 2003; Murphy and Tranel 2019).

Target site resistance was first confirmed in smooth pigweed (*Amaranthus hybridus* L.) to the PSII inhibitor atrazine (WSSA Group 5) in 1983 as the result of a point mutation changing serine-264 to glycine (Hirshberg and Mcintosh 1983). More recently, target site resistance to 2,4-dichlorophenoxyacetic acid (2,4-D) (WSSA Group 4) has been confirmed in kochia (*Bassia scoparia* (L.) A. J. Scott) in 2018 as the result of a two-nucleotide substitution changing glycine-73 to asparagine (LeClere et al. 2018). Herbicide resistance factors (RF) are determined by taking the dose of an herbicide required to reduce growth of a resistant population by 50% and dividing it by the dose required to reduce the growth of a susceptible population by 50%. Target site resistance via altered target sites often result in high RF. Certain weed biotypes that have developed resistance to triazine herbicides have been found to have an RF of 1,000 at the target site, while the whole plant has an RF of 100 (Devine and Shukla 2000). Likewise, populations of

Palmer amaranth and common waterhemp have been found to demonstrate extremely high resistance levels to ALS inhibiting herbicides. Sprague et al. (1997) found that resistant biotypes of Palmer amaranth and common waterhemp exhibited resistance to imazethapyr that was greater than 2,800-fold and 130-fold, respectively, compared with a susceptible biotype. Target site resistance to glyphosate is unique when compared with other herbicides. In order to confer strong resistance to glyphosate, multiple amino acid substitutions within the target site are necessary (Murphy and Tranel 2019). Single amino acid substitutions can result in glyphosate resistance of 2-6x, while substitutions of two amino acids often results in resistance factors upwards of 180x (Heap and Duke 2018). Single base pair mutations providing resistance to glyphosate typically result in changes of Pro106 and have been documented in goosegrass (*Eleusine indica* (L.) Gaertn.), rigid ryegrass (*Lolium rigidum* Gaudin), Italian ryegrass (*Lolium perenne spp. multiflorum*), and common waterhemp (Heap and Duke 2018). Double base pair alterations in Thr102 and Pro106 have been used to convey glyphosate-resistance in commercial corn (*Zea mays* L.) varieties; this double mutation has also been found in goosegrass (Heap and Duke 2018).

Even though target site alterations are the most common mechanism for target site resistance to an herbicide, gene amplification has been confirmed as the mechanism of resistance for both ACCase inhibitors (Group 1) and the EPSPS inhibitor glyphosate (Group 9) (Gaines et al. 2010; Laforest et al. 2017). Amplification of the EPSPS gene has been associated with glyphosate resistance in nine weed species including: Palmer amaranth, waterhemp, spiny amaranth (*Amaranthus spinosus* L.), Italian ryegrass, Kochia, ripgut brome (*Bromus diandrus* Roth.), goosegrass, and tall windmill grass (*Chloris truncate* R. Br.) (Heap and Duke 2018). Glyphosate-resistance via gene amplification often results in RF (6-10x) that are slightly greater than those involving single mutations, such as Pro106, of the target site (2-6x) (Gaines et al. 2010; Heap and Duke 2018).

Non-target site resistance can broadly be defined as, "…any mechanism that reduces the amount of herbicide that reaches the target site, or that ameliorates the effect of the herbicide despite its inhibition of the target site" (Murphy and Tranel 2019). Non-target site resistance mechanisms include enhanced metabolism, reduced absorption/translocation, and herbicide sequestration into vacuoles. Glyphosate-resistance as a result of non-target site mechanisms have been confirmed and often confer 3-12 fold resistance (Heap and Duke 2018). Enhanced metabolism has been used to confer glyphosate-resistance in canola (*Brassica napus* L.) and is also responsible for the natural tolerance of pitted morningglory (*Ipomoea lacunosa* L.) to glyphosate (Correa et al. 2016; Ribiero et al. 2015). Physiological changes to the leaf shape, cuticle, or epidermis can result in reduced interception or uptake of an herbicide into a plant. Reduced absorption conferring glyphosate resistance has been confirmed in johnsongrass (*Sorghum halepense* (L.) Pers.), Italian ryegrass, sourgrass (*Digitaria insularis* (L.) Mez ex Ekman), tall windmill grass, and Judd's grass (*Leptochloa virgata* (L.) P. Beauv.) (Heap and Duke 2018). Reduced translocation of glyphosate from plant leaves to meristems has been reported in tall windmill grass, hairy fleabane (*Erigeron bonariensis* L.), horseweed, rigid ryegrass, and Italian ryegrass (Brunharo et al. 2016; Feng et al. 2004; Shaner 2009). In horseweed and certain *Lolium* spp., rapid sequestration of glyphosate into the vacuole of the plant cell has been deemed responsible for reduced translocation within the plant (Sammons and Gaines 2014). Reduced translocation in giant ragweed has been documented as a result of the 'Phoenix phenomenon,' where treated leaves rapid desiccate, preventing the translocation of glyphosate throughout the plant (Moretti et al. 2017; Van Horn et al. 2017).

Economically Important Glyphosate-Resistant Weeds

Palmer amaranth. Palmer amaranth is dioecious summer annual species that is indigenous to the Sonoran Desert in the Southwestern United States and has established itself as the most successful weedy species of the *Amaranthus* family (Ehleringer 1983; Sauer 1957). Palmer amaranth has become one of the most troublesome weeds in the United States. Palmer amaranth was first observed in South Carolina in 1989 and within 6 years was deemed the most problematic weed in cotton (*Gossypium hirsutum* L.) production and one of the top five most problematic weeds in soybean production (Webster and Coble 1997). In 2009, Palmer amaranth was ranked in the top 10 most troublesome weeds in corn, soybean, and cotton across many southern states (Webster and Nichols 2012). Palmer amaranth has continued to expand northward, spreading into Midwestern states (Sellers et al. 2003) including Michigan (Sprague 2011).

Germination of Palmer amaranth occurs rapidly under warm temperatures. Although it has been reported that germination can occur at minimum temperatures of 17° C (Steinmaus et al. 2000); peak germination has been observed with alternating temperatures of 32 to 38° C (Guo and Al-Khatib 2003). Once germinated, emergence of Palmer amaranth seedlings can occur within 5 days (Sellers et al. 2003). Palmer amaranth has the ability to germinate and emerge throughout the growing season. In Michigan, emergence of Palmer amaranth has been reported to occur from mid-May through September in non-crop conditions (Powell 2014). Palmer amaranth has the greatest emergence when seed germinates in the top 1.3 cm of soil (Keeley et al. 1987). Jha and Norsworthy (2009) found that emergence of Palmer amaranth can be reduced upwards of 70% under a developed soybean canopy.

Other biological characteristics of Palmer amaranth, including its rapid growth rate, biomass accumulation, and prolific seed production, add to this weed's highly competitive nature. Even though Palmer amaranth can emerge throughout the growing season, it still has the capability to acquire large quantities of biomass as a result of its rapid growth rate (Jha and Norsworthy 2009). Horak and Loughin (2000) found that Palmer amaranth grew at a quicker rate, 0.21 cm GDD^{-1}, and accumulated more biomass, 0.32 g g^{-1} day^{-1}, than other *Amaranthus* species. As a tall, upright plant, Palmer amaranth is typically 2 m in height but can grow upwards of 3 m tall under optimal growing conditions (Sauer 1955). Sellers et al. (2003) found Palmer amaranth to be 45% taller than common waterhemp and 600% taller than redroot pigweed. Seed production rates for Palmer amaranth are highly dependent on the climate and time of establishment in an ecosystem. When grown in the absence of plant competition, Palmer amaranth seed production was 250,000, 446,000, and 613,000 seeds per plant in Missouri, Georgia, and California, respectively (Keeley et al. 1987; Sellers et al. 2003; Webster and Grey 2015). As Palmer amaranth emerges later in the growing season, seed production decreases.

Palmer amaranth reduces soybean yield by directly competing for water, light, and nutrients. Klingaman and Oliver (1994) reported Palmer amaranth densities of 0.33 to 10 plants m^{-1} of row reduced soybean yield by 17 to 64%, respectively. Similarly, Bensch et al. (2003) found that soybean yield was reduced by 79% when Palmer amaranth emerged at a similar time as soybean at a density of 8 plants m^{-1} of row. The competitiveness of Palmer amaranth is greater in warmer climates compared with cooler climates, as photosynthetic rates are correlated with temperature. As a result, Palmer amaranth may not be able to able to effectively compete with crops and other weeds in Michigan's cooler climate. Ehleringer (1983) found that 90% of the peak photosynthetic rate for Palmer amaranth occurred between 36-46° C while temperatures of

25° C provided a maximum photosynthetic rate of 50%. The effects of Palmer amaranth on crop production are minimized with delayed emergence, likely as a result of increased shading. However, Jha et al. (2008) demonstrated that Palmer amaranth can exhibit a shade avoidance response where leaf area is altered, and chlorophyll content increased under shaded conditions to maintain growth.

Waterhemp. Waterhemp is a dioecious summer annual weed that is indigenous to the Midwestern United States (Wax 1995). In the past, two species of waterhemp were recognized: tall waterhemp (*A. tuberculatus* (Moq.) J.D. Sauer) and common waterhemp (*A. rudis* Sauer) with the only differentiation between species being their geographic origin (Waselkov and Olsen 2014). However, expansion of each species into the other's geography has resulted in hybridization between species with progeny that are unable to be identified as either parent species (Pratt and Clark 2001). For this reason, the two species have generally been merged into one, waterhemp, *Amaranthus tuberculatus* (Moq.) J.D. Sauer.

Germination of waterhemp occurs rapidly under optimal soil temperatures and moisture. However, germination of waterhemp has been noted to occur throughout the growing season. Froud-Williams et al. (1984) proposed that initial germination of waterhemp in the spring is the result of warming soil temperatures with successive germination events occurring later in the season being reliant on rainfall events. Likewise, Refsell and Hartzler (2009) observed waterhemp germination and emergence occurring in mid-July following significant rainfall. Studies explicitly examining waterhemp germination have found that minimal emergence occurs when soil temperatures are below 20° C (Guo and Al-Khatib 2003; Steckel et al. 2004) while field studies have reported waterhemp germination and emergence with soil temperatures of 12°

C (Steckel et al. 2007). Once soil temperatures reach 35° C, germination rapidly declines, and germination does not occur at soil temperatures above 48° C (Steckel et al. 2004). Compared with other summer annual weeds, waterhemp was consistently the last to emerge and had a mean duration of emergence 53 days in length (Hager et al. 1997; Hartzler et al. 1999). Leon and Owen (2006) observed waterhemp germination after 70 days in no-till conditions. Waterhemp's delayed and prolonged emergence into the growing season is one of its most problematic biological traits for crop production systems.

Like Palmer amaranth, waterhemp's rapid growth rate, biomass accumulation, and immense seed production add to the weed's highly competitive nature. Horak and Loughin (2000) found that waterhemp growth patterns were slightly lower than those of Palmer amaranth with vertical growth occurring at a rate upwards of 0.16 cm GDD^{-1} and biomass accumulation occurring at a rate of 0.30 g g^{-1} day^{-1}. When emerging with soybean, waterhemp has been observed to reach approximately 2 m in height (Hartzler et al. 2004). Although waterhemp's growth rate is second only to Palmer amaranth, Sellers et al. (2003) observed that waterhemp was the fourth tallest species of six different *Amaranthus spp*. studied. Seed production rates for waterhemp vary based on the time of emergence and environmental conditions during the growing season. Sellers et al. (2003) reported an average seed production of just under 300,000 seeds $plant^{-1}$ when intra- and interspecies competition was eliminated. Similarly, Hartlzer et al. (2004) observed an average seed production of 309,000 seeds $plant^{-1}$ when waterhemp emerged at the same time as soybean. Under optimal conditions, Steckel et al. (2003) observed seed production over one million seeds per plant. Delays in emergence significantly reduce overall seed production, as plants emerging 50 days after soybean planting only produced 3,000 seeds plant^{-1} (Hartzler et al. 2004). Steckel and Sprague (2003) found that seed production was similar across both wide and narrow soybean row widths.

Waterhemp's rapid growth rate and biomass accumulation allow it to effectively compete with agronomic crops. Bensch et al. (2003) found that soybean yields were reduced by 56% when waterhemp emerged at a similar time as soybean at a density of 8 plants m^{-1} of row. Similarly, Hager et al. (2002) found that if waterhemp emerged with soybean and was left uncontrolled for the growing season that soybean yield losses of 43% could occur. Likewise, Hager et al. (2002) reported that waterhemp controlled within two weeks of unifoliate expansion was not detrimental to soybean yield; however, negative effects on soybean yield were observed if waterhemp was left unmanaged for four weeks following unifoliate expansion. Similarly, Hartzler et al. (2004) reported that waterhemp emerging after the V4 growth stage in soybean are unlikely to have an impact on crop yield as a result of reduced growth and high mortality.

Horseweed. Horseweed is a facultative winter annual weed species that is native to North America and is capable of growing in a multitude of environments from roadsides to crop production fields (Weaver 2001). As a facultative winter annual, horseweed typically emerges in the fall following declining soil temperatures; however, horseweed can also emerge facultatively at other times of the year (Cici and Van Acker 2009). Environmental conditions that favor germination of horseweed are extensive (Nandula et al. 2006). Steinmaus et al. (2000) determined the base temperature for horseweed germination to be 13° C, intermediate to that of summer and winter annuals. Horseweed germination increases with temperature, as peak germination occurred with day/night temperatures of 24/20° C (Nandula et al. 2006). No germination occurred at day/night temperatures of 12/6° C (Nandula et al. 2006). Depth of seed burial also influences germination. Tremmel and Peterson (1983) observed reduced horseweed germination from a depth of 1 cm compared with seed sown on the soil surface. Similarly, Nandula et al. (2006) found peak germination occurs from the soil surface with no germination occurring at seed burial depths of 0.5 cm or greater. Horseweed germination is typically highest in well-drained, coarse textured soils (Nandula et al. 2006). Although germination rates are typically higher following rain events or where soil moisture is high, horseweed has minimal tolerance to flooding (Regehr and Bazzaz 1979; Smith and Moss 1998; Stoecker et al. 1995). Peak emergence of horseweed typically occurs in April to June and August to October, but emergence throughout the growing season has been observed (Bhowmik and Bekech 1993; Buhler and Owen 1997; Loux et al. 2006; Main et al. 2006). Davis and Johnson (2008) observed over 90% of horseweed emergence in the spring in Indiana. In contrast, Buhler and Owen (1997) reported that only 5 to 19% and 28 to 32% of total horseweed emergence occurred in the spring for populations in Iowa and Minnesota, respectively.

Horseweed flowering typically occurs in July with peak seed production occurring in the months of August and September (Weaver 2001). Genes responsible for vernalization in winter wheat (*Triticum aestivum* L.) and *Arabidopsis thanliana* (L.) Heynh. are present in horseweed (He et al. 2004; Rudnoy et al. 2002) which indicates that cold periods may be required for flower production. However, spring germinated horseweed is still able to flower and produce seed (Regehr and Bazzaz 1979). Horseweed seed takes approximately three weeks to mature following successful fertilization (Weaver 2001). In the absence of a crop or at low plant densities, each horseweed plant is capable of producing 200,000 seeds (Bhowmik and Bekech 1993). Increasing plant density negatively effects horseweed seed production (Bhowmik and Bekech 1993; Palmblad 1968). Time of emergence also influences seed production. Regehr and

Bazzaz (1979) found that fall emerged plants produced more seeds than that of those emerged in the spring. Seed production is also greater in taller plants than in shorter plants (Regehr and Bazzaz 1979). Seed dispersal typically occurs via wind (Weaver 2001). Tall stems and the attachment of a pappus to horseweed seed are adaptations for wind dispersal (Weaver 2001). Dauer et al. (2007) found that horseweed seed regularly disperses 500 m from its source; however, 99% of the seed remains within 100 m from its source. Viable seed has been found more than 50 m above ground in the Planetary Boundary Layer (PBL) (Dauer et al. 2009; Shields et al. 2006). Once in the PBL, seed travel greater than 500 km is possible due to low turbulence, high wind speed, and greater laminar flow (Shields et al. 2006).

Horseweed has a relatively low relative growth rate compared with other economically important glyphosate-resistant weeds like Palmer amaranth and common waterhemp. Levang-Brilz and Biondini (2002) reported horseweed to have a relative growth rate of 0.16 g g^{-1} day^{-1}. Similarly, Davis et al. (2009) reported a mean relative growth rate of 0.157 g g^{-1} day^{-1} across four horseweed populations. Horseweed has a similar relative growth rate compared with soybean, which has been reported to be 0.155 g g^{-1} day^{-1} (Seibert and Pearce 1993). Horseweed is believed to be less competitive than most other summer annual weed species (Loux et al. 2006). Limited studies examining horseweed competition with crops exist. Prior to the development of herbicide resistance, soybean yield was reduced by 83% when horseweed was left unmanaged (Bruce and Kells 1990). Following the development of glyphosate-resistance, untreated horseweed has resulted in soybean yield loss up to 97% compared with varying burndown herbicide programs (Eubank et al. 2008). Similarly, soybean yield reductions of 83 to 93% have been reported when glyphosate-resistant horseweed has been left unmanaged (Byker et al. 2013).

New Technologies for Glyphosate-Resistant Weed Control in Soybean

The introduction of glyphosate-resistant crops along with the simple and effective broad-spectrum weed control from the use of glyphosate in crop reduced the urgency for discovering and developing new herbicide modes of action. From the time period surrounding the 1950s to the 1980s, herbicide discovery occurred at an astounding rate in which a novel site of action was discovered roughly every 2 years (Dayan 2019). Although the implementation of GR crops has proved to play a major role in the lapse of herbicide discovery, the issue is only compounded by the increased cost for research and development. In 2000, the estimated cost to produce a single active ingredient was $184 million compared with $286 million in 2016 (McDougall 2016). The HPPD inhibiting herbicides (WSSA Group 27), known for their bleaching symptomology, were first commercialized for use in rice (*Oryza sativa* L.) in 1980 (Cole et al. 2000). However, it wasn't until the 1990s that the HPPD inhibitors were recognized and pursued as the most recent novel site of action with the introduction of the herbicides mesotrione and isoxaflutole (Pallet 2000).

Currently, 50 different weed species around the globe have developed resistance to glyphosate, with 17 of these species present in the United States (Heap 2020). Several of these GR weed species, including horseweed, common waterhemp, Palmer amaranth, and giant ragweed, are problematic across a variety of cropping systems throughout the United States, including Michigan (Heap 2020; Powles 2008). The emergence and continual development of glyphosate- and multiple-resistant weeds paired with the prolonged drought in discovering novel herbicide modes of action has influenced the development and commercialization of new herbicide-resistant traits in field crops, specifically glufosinate, dicamba, 2,4-D, and isoxaflutole resistance in soybean (Behrens et al. 2007; Green and Castle 2010; Nandula 2019; Powles 2008;

Wright et al. 2010). The engineered resistance of soybean to these herbicides, which they are naturally sensitive to, would allow for increased control of GR weeds in soybean.

Following the stable insertion of the *pat* gene and CaMV 35S promoter, glufosinate-resistant soybean, sold under the trade name LibertyLink® (BASF, Research Triangle Park, NC), were commercialized in 2009 to combat GR weeds such as Palmer amaranth and common waterhemp (Green and Castle 2010). At the time, only one glufosinate product was formulated and marketed for use in this system, sold under the trade name Liberty® (BASF, Research Triangle Park, NC). In 2019, glufosinate-resistant soybeans were responsible for 20% of the United States soybean market share (Nandula 2019). Other glufosinate-resistant crops include canola (1995), corn (1997), cotton (2004); rice, sugarbeet (*Beta vulgaris* L.), and wheat have also been engineered with resistance to glufosinate but are not currently commercially available (Takano and Dayan 2020).

The insertion of the dicamba monooxygenase enzyme from the soil bacterium, *Pseudomonas maltophilia* (strain DI-6), into soybean resulted in conferred resistance to the herbicide dicamba (Behrens et al. 2007). The dicamba-resistant soybean, marketed under the name Roundup Ready 2 Xtend® (Bayer Crop Science, St. Louis, MO), are also resistant to glyphosate. The Roundup Ready 2 Xtend® soybean was deregulated by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) in 2015, allowing for commercialization during the 2016 crop year. However, low volatility dicamba formulations developed for use with these soybeans were not registered for POST use in soybean by the United States Environmental Protection Agency (EPA) until the middle of the 2016 growing season. This initial registration was valid through December of 2018 (US-EPA 2020). PRE applications of dicamba are also approved for use in dicamba-resistant soybean. The EPA approved dicamba herbicides for use in Roundup Ready 2 Xtend® soybean are marketed under the trade names of Xtendimax® (Bayer Crop Science, St. Louis, MO), FeXapan® (Corteva Agriscience, Wilmington, DE), and Engenia® (BASF, Research Triangle Park, NC). An additional dicamba product, formulated with S-metolachlor, was registered in 2019 for use in soybean. This new herbicide is sold under the trade name Tavium® (Syngenta, Research Triangle Park, NC). The registration of dicamba for POST applications in soybean was extended in 2018 until December of 2020. Following a lawsuit, the US Ninth Circuit Court of Appeals voted to vacate EPA registrations for Xtendimax®, FeXapan®, and Engenia® after July 31, 2020. However, in October of 2020, the EPA announced the reregistration of these dicamba products for use on crops resistant to dicamba through December of 2025 (US-EPS 2020).

Similarly, the insertion of the aryloxyalkanoate dioxygenase enzyme AAD-12 from the bacterium *Delftia acidovorans* into soybean resulted in resistance to 2,4-D (Wright et al. 2007, 2010). 2,4-D-resistant soybean, marketed as Enlist E3® (Corteva Agriscience, Wilmington, DE), also conferred resistance to glyphosate and glufosinate as the result of a single molecular stack containing all three resistance genes (Nandula 2019). A new, low volatility formulation of 2,4-D was developed for use in Enlist E3® soybean. This new 2,4-D product is formulated using Colex-D® technology (Corteva Agriscience, Wilmington, DE). This new 2,4-D is formulated as a choline salt, which has lower volatility than traditional 2,4-D ester or amine formulations (Sosnoskie et al. 2015). 2,4-D choline is sold under the trade name Enlist One® (Corteva Agriscience, Wilmington, DE) and as a premixture with glyphosate as Enlist Duo® (Corteva Agriscience, Wilmington, DE). Both PRE and POST applications of 2,4-D choline, as well as glufosinate can be made to Enlist E3® soybean.

In 2019, LibertyLink® GT27™ (BASF, Research Triangle Park, NC) soybean was released for commercial use. This new soybean system builds on the LibertyLink® platform released a decade earlier. This soybean technology offers growers the flexibility to apply three herbicides: glyphosate, glufosinate, and the HPPD inhibitor (Group 27) isoxaflutole (Smith et al. 2019). Isoxaflutole registered for use in soybean is sold under the trade name Alite 27® (BASF, Research Triangle Park, NC) and received its registration from the US EPA in 2020. However, isoxaflutole is only registered for preemergence use in LibertyLink® GT27™ soybean planted in select counties within 25 US states (Anonymous 2020).

Currently, Bayer Crop Science has built upon the Roundup Ready 2 Xtend® soybean platform by successfully breeding the *pat* gene into the Roundup Ready 2 Xtend® soybean, allowing for additional herbicide resistance to glufosinate (ISAAA 2020). This new technology is being marketed as XtendFlex® (Bayer CropScience, St. Louis, MO) soybean and is currently in the process of becoming commercialized in 2021. XtendFlex® soybean provides growers with the flexibility to apply dicamba and/or glufosinate for PRE or POST control of glyphosate-resistant weeds, such as horseweed, Palmer amaranth, and waterhemp. Only EPA approved formulations of dicamba may be used for use in XtendFlex® soybean, including Engenia®, FeXapan®, Tavium®, and Xtendimax®.

Glufosinate- and Dicamba-Based Weed Control

Glufosinate has proven to be effective in controlling a variety of annual broadleaf weeds but has its limitations on controlling grass species (Haas and Muller 1987; Steckel et al. 1997; Thomson 1993). Although highly effective on most broadleaf species, common lambsquarters is not very sensitive to glufosinate (Takano and Dayan 2020). Glufosinate is effective among a variety of species; however, variation in tolerance to glufosinate across species exists (Haas and Muller 1987). One of the most important factors influencing weed control with glufosinate is weed size. Steckel et al. (1997) reported that the control of four different species was reduced 49% when glufosinate was applied at 15 cm tall plants versus 10 cm tall plants. However, Meyer and Norsworthy (2019) reported a reduction in Palmer amaranth control of only 9% five weeks after application when delaying application timing from 10 cm to 30 cm.

Glufosinate has proven to be effective in managing glyphosate-resistant horseweed in early preplant (EPP) applications, although plant density and the interval between herbicide application and planting results in variation of control (Eubank et al. 2008; Steckel et al. 2006). Horseweed control with glufosinate is optimized by applications made midday and under ideal environmental conditions (Montgomery et al. 2017). Glufosinate is also effective in controlling common waterhemp and Palmer amaranth. Glufosinate applied at 0.29 and 0.40 kg ai ha^{-1} provided greater than 99% control of common waterhemp 4 weeks after application (Beyers et al. 2002). Similarly, Coetzer et al. (2002) reported that glufosinate provided control of common waterhemp up to 91%. Common waterhemp control was greater than Palmer amaranth control when utilizing a single POST application of glufosinate at 2-, 4-, and 8-weeks after application (Coetzer et al. 2002). However, Jhala et al. (2017) demonstrated the importance of PRE herbicides in order to achieve acceptable control of common waterhemp when utilizing glufosinate POST.

Commercialization of the Roundup Ready 2 Xtend® soybean has allowed for postemergence use of dicamba for managing tough to control broadleaf weeds. In this system, dicamba can also be used EPP for the effective burndown of winter annual species. Dicamba has been reported to have moderate soil residual activity (Shaner 2014c). When applied preemergence, Johnson et al. (2010) reported the residual activity of dicamba provided sufficient early-season control of glyphosate-resistant horseweed. When used in a burndown application, Kruger et al. (2010) reported that the size of horseweed did not influence the efficacy of dicamba. Similar findings have been reported by Byker et al. (2013), where dicamba applied preplant or in sequential applications provided greater than 90% control of horseweed 8 weeks after treatment. Merchant et al. (2013) reported that dicamba applied at 0.56 kg ae ha^{-1} provided 76% control of glyphosate-resistant Palmer amaranth. Spaunhorst and Bradley (2013) reported poor control of glyphosate-resistant common waterhemp (22% to 27% control) when dicamba was applied at 0.56 kg ae ha^{-1}.

Although rare and only documented in a limited number of weed species resistance to glufosinate has been documented. In the majority of these instances, the mechanism of resistance is not well understood (Takano and Dayan 2020). Prior to 2020, glufosinate-resistance in weeds had only been documented in four grass species, including goosegrass, rigid ryegrass, perennial ryegrass (*Lolium perenne* L.), and Italian ryegrass; the first of such cases occurred in a Malaysian population of goosegrass in 2009 (Heap 2020; Jalaludin et al. 2010). In this population, the mechanism for resistance was not associated with an altered target site, increased metabolism, or changes in absorption or translocation of the herbicide (Jalaludin et al. 2017). Glufosinate-resistance has been documented in the United States. Italian ryegrass populations located in orchards from Oregon and California were confirmed to be resistant to glufosinate in 2010 and 2015, respectively (Heap 2020). Enhanced metabolism of glufosinate has been associated as the mechanism of resistance for Italian ryegrass from Oregon (Brunharo et al. 2019). A separate population of Italian ryegrass from Oregon has also been confirmed resistant to glufosinate as a result of a target site mutation (Avila-Garcia et al. 2012). Although not regarded as resistant at the time, a population of Palmer amaranth from Arkansas has shown reduced sensitivity to glufosinate coupled with an increase in expression of the GST and cytochrome P450 monooxygenase detoxification enzymes (Salas-Perez et al. 2018). More recently, in February of 2021, the University of Arkansas released an extension article describing a population of Palmer amaranth that was confirmed to be resistant to field rates of glufosinate following failures in control during in 2020 (Barber et al. 2021). However, this information has not been published into current scientific literature.

The development of resistance to dicamba in weeds has also been documented. Currently nine species have developed resistance to dicamba with the first case of resistance occurring in wild mustard from Manitoba, Canada in 1990 (Heap 2020). It is believed that altered binding of dicamba to its target site in this population is responsible for the development of resistance (Deshpande and Hall 2000). Resistance to dicamba in the United States is limited to three species, kochia, prickly lettuce (*Lactuca serriola* L.), and Palmer amaranth, with the first case occurring in 1994 in a population of kochia located in Montana (Heap 2020). Resistance to dicamba in kochia within the United States has been documented eight unique times in which differences in the uptake, translocation, or metabolism of the herbicide did not occur (Cranston et al. 2001). However, Pettinga et al. (2017) found that reduced translocation of dicamba was in fact responsible for the development of resistance among one population of kochia along with the increased expression of the chalcone synthase enzyme. The mechanism of resistance for prickly lettuce has not been evaluated; however, prickly lettuce is only cross-resistant to dicamba as a result of resistance to 2,4-D (Burke et al. 2009). More recently, a Tennessee population of Palmer amaranth has been confirmed resistant to dicamba through greenhouse dose-response experiments (Heap 2020).

Characteristics of Glyphosate, Glufosinate and Dicamba

Glyphosate. Glyphosate [N-(phosphonomethyl)glycine] is a member of the organophosphorus chemical family and has been classified as an amino acid synthesis inhibitor. Specifically, glyphosate is classified as an inhibitor of enolpyruvyl shikimate-3-phosphate synthase (EPSPS) under the WSSA Group 9 SOA and is the only such chemical in this SOA (Mallory-Smith and Retzinger 2003; Shaner 2014a). The herbicidal effects of glyphosate were first identified in 1970 and in 1974 the first formulated glyphosate product was commercially released, providing non-selective and broad-spectrum weed control (Duke and Powles 2008). At this point in time, glyphosate's use was primarily limited to directed POST applications in high-value horticultural crops as well as POST applications in some non-crop situations as a result of its expensive price point (Heap and Duke 2018). The effectiveness of glyphosate has proven to be unmatched as it controls more weed species than any other herbicide (Heap and Duke 2018).

Prior to the introduction of GR crops, glyphosate was used for vegetation management across a variety of systems. Applications of 0.21 – 2.24 kg ae ha^{-1} could be made EPP or PRE in no-till systems to control emerged weeds prior to planting or crop emergence (Shaner 2014a). Postemergence uses of glyphosate prior to the release of GR crops were limited to non-crop areas, such as industrial sites, railways, other rights-of-way, and anywhere else where total vegetation control was desired; applications rates ranged from 0.84 – 4.2 kg ae ha^{-1} (Benbrook 2016; Shaner 2014a). Directed POST applications of 0.84 – 4.2 kg ae ha^{-1} were made in tree and vine crops as well as in ornamental crops and Christmas trees (Shaner 2014a). Glyphosate was also used in preharvest applications of 0.84 – 4.2 kg ae ha^{-1} in cotton and 0.21 – 0.84 kg ae ha^{-1} in wheat to assist with the desiccation of both crops (Shaner 2014a). The control of woody species with glyphosate was achieved with injection or by treating freshly cut stumps (Shaner

2014a). In GR crops, POST applications of 0.35 – 0.70 kg ae ha^{-1} can be made in accordance with the label (Anonymous 2020b). Overall, the non-selective nature of glyphosate allows for its control of nearly all annual and perennial vegetation. However, glyphosate is especially effective in controlling annual grasses. Glyphosate's use in the United States has exponentially increased since its commercial release. From 1974 to 2014, the use of glyphosate in the agricultural sector increased by a factor of 315X (Benbrook 2016).

Glyphosate is classified as a weak acid with a pKa value of 2.6, a K_{ow} of 0.0006 – 0.0017, and a K_{oc} of 24,000 mL g^{-1} (Shaner 2014a). In the acid form, glyphosate is hydrophilic with a logK_{ow} of -3.2 to -2.8 and a solubility in water of 15,700 mg L^{-1}. When formulated as the isopropylamine or trimethylfulfonium (TMS) salts, glyphosate's solubility in water increases to 900,000 mg L^{-1} and 4,300,000 mg L^{-1} respectively (Shaner 2014a). A high K_{oc} value of 24,000 mL g^{-1} significantly reduces the ability of glyphosate to leach through the soil profile (Shaner 2014a). Once applied, glyphosate is rapidly bound to soil particles and varying soil properties have minimal effects on adsorption (Shaner 2014a). It has been reported that glyphosate adsorption to the soil is directly correlated with the amount of vacant phosphate sorption sites (Hance 1976; Sprankle et al. 1975a). This rapid and tight adsorption of glyphosate molecules to soil particles minimizes phytotoxic effects in soil-applied applications, limiting glyphosate's uses to foliar applications only. Although strongly bound to the soil, glyphosate is moderately persistent with a half-life of 47 days (Wauchope et al. 1992). However, due to the strong sorption of glyphosate molecules to the soil, all crops can be planted immediately after an application. Degradation of glyphosate within the soil occurs through microbial interactions with upwards of 70% of applied glyphosate metabolized into CO_2 over the course of a growing season; non-microbial degradation of glyphosate in the soil is negligible (Shaner 2014a). With a vapor pressure of 2.45 x 10^{-8} Pa and 3.99 x 10^{-5} Pa at a 25º C, neither glyphosate acid or glyphosate formulated as the TMS salt are volatile (Shaner 2014a).

Classified as a systemic herbicide, glyphosate molecules are capable of translocating throughout the plant in the symplast and accumulate in areas of active growth including meristems, immature leaves, and roots (Martin and Edgington 1981). Moderate absorption of glyphosate across the plant cuticle is observed from POST applications (Boerboom and Wyse 1988; Coupland 1984; Marshall et al. 1987). Commercial formulations of glyphosate are more readily absorbed into the affected plant than is glyphosate in the acid form (MacIsaac et al. 1991). The addition of nonionic surfactant as well as ammonium sulfate to spray solutions increase absorption across the cuticle (MacIsaac et al. 1991; Sprankle et al. 1975b; Thelen et al. 1995). Once absorbed into the plant, the transport of absorbed glyphosate molecules across the plasma membrane and into plant cells occurs in a relatively slow manner (Jachetta et al. 1986). It is believed that a phosphate transporter may assist in the movement of glyphosate molecules across the plasma membrane and into plant cells (Denis and Delrot 1993). Characteristic symptoms of affected plants include the stalling of plant growth shortly after application as well as gradual chlorosis of young plant tissue followed by necrosis of the entire plant (Shaner 2014a). Plant death occurs quicker in annual grasses than in annual broadleaves or perennials. Full control of grasses typical occurs in 7 days and control of annual broadleaves or perennials often takes 10 – 20 days (Shaner 2014a).

Glyphosate is characterized as an amino acid synthesis inhibitor. More specifically, glyphosate is characterized by the WSSA Group 9 SOA as an inhibitor of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). EPSPS is a critical enzyme in the Shikimate pathway of plants which allows for the production of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, which are needed for protein synthesis and other biosynthetic pathways (Shaner 2006). Plants are unable to source these amino acids from external sources and as a result, rely solely on biochemical pathways to produce acceptable quantities. Previous studies have found that the addition of these amino acids with an application of glyphosate still resulted in toxicity to plants, suggesting that other pathways besides the inhibition of protein synthesis are inhibited (Duke and Hoagland 1978; Lee 1980). Although glyphosate is nearing 50 years of use as an herbicide, its entire mode of action is not fully understood.

Glufosinate. Similar to glyphosate, glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid] is a member of the organophosphorus chemical family and has been classified as a nitrogen metabolism inhibitor; glufosinate is the only active ingredient in the WSSA Group 10 SOA (Mallory-Smith and Retzinger 2003; Shaner 2014b). Glufosinate was commercialized in 1993 as an herbicide providing non-selective and broad-spectrum weed control (Takano and Dayan 2020). At that time, the herbicide's primary purpose was for burndown uses in EPP applications as well as POST applications in non-crop areas or directed POST applications in nursery stock at rates of 0.35 – 1.7 kg active ingredient (ai) ha^{-1} (Shaner 2014b). Today, applications of 0.59 – 0.79 kg ai ha^{-1} can be made POST in glufosinate-resistant canola, corn, cotton, and soybean (Anonymous 2020c). Prior to 2014, use of glufosinate remained stable across the United States. However, the rapid development and spread of glyphosate-resistant weed species coupled with the introgression of glufosinate-resistance into soybean varieties resulted in glufosinate use tripling from 2014 to 2017, where almost 6.5 million kg of glufosinate were used (USGS 2020).

Glufosinate is a weak acid with varying dissociation constants as a result of the amine and hydroxyl groups present in its chemical structure; glufosinate has pKa values of 2, 2.9, and

9.8 (Shaner 2014b; Takano and Dayan 2020). As an unformulated product, glufosinate is an extremely hydrophilic compound with a $\log K_{ow}$ of –4.0 and a solubility in water of 1,370,000 mg L^{-1}. With a relatively low K_{oc} of 100 mL g^{-1} coupled with the compound's hydrophilic property, the leaching potential of glufosinate through the soil is very high (Shaner 2014b). Although highly mobile in soil, glufosinate has never been found deeper than 15 inches into the soil profile (Shaner 2014b). Glufosinate is not very persistent with a half-life of 7 days and rapid degradation in both soil and water occurring via microbial activity into one primary metabolite and ultimately to CO_2 (Shaner 2014b). With a vapor pressure of 10^{-4} Pa at a 25° C, glufosinate is not volatile. Glufosinate is commercially available as its ammonium salt under a multitude of trade names.

Classified as a contact herbicide, applications of glufosinate require excellent coverage across plant biomass to achieve optimal control. Translocation of glufosinate throughout a treated plant is limited and little to no absorption occurs through the roots (Shaner 2014b). Absorption of glufosinate is optimized by warm temperatures, high humidity, and bright sunlight; a rain-free period of 4 hours is required following treatment (Anonymous 2020c; Coetzer et al. 2001; Shaner 2014b; Takano and Dayan 2020; Takano et al. 2020). To optimize sunlight conditions, glufosinate may only be sprayed from dawn to 2 hours before sunset (Anonymous 2020c). Characteristic symptoms of affected plants include rapid chlorosis and necrosis of plant tissue, as well as suppression of plant growth (Takano and Dayan 2020). Optimal environmental conditions increase effectiveness of glufosinate applications and necrosis is observed in 2-4 days with plant death occurring in 10-14 days (Anonymous 2020c; Shaner 2014b). As a contact herbicide, glufosinate provides the greatest control of small weeds, typically 10 cm or smaller (Steckel et al. 1997). Although glufosinate acts as a non-selective, broad-spectrum herbicide, its performance is greater on annual broadleaf species than on grasses and perennials (Steckel et al. 1997; Tharp et al. 1999).

As characterized by the WSSA Group 10 SOA, glufosinate acts as a nitrogen metabolism inhibitor. Specifically, glufosinate inhibits the activity of glutamine synthetase, which is the second most abundant protein in plant leaves (Takano and Dayan 2020). Glutamine synthetase is essential for nitrogen metabolism within plants by using energy to incorporate ammonia and glutamate to yield glutamine, an important amino acid (Bernard and Habash 2009). Glufosinate is a potent and irreversible inhibitor of glutamine synthetase in plant species and acts as a direct competitor with glutamate for the active site (Forlani et al. 2006). Once glufosinate binds to the active site, rapid accumulation of ammonia occurs within the plant, destroying cells and inhibiting reactions within photosystems I and II (Wild et al. 1987). Mutagenesis within the active site could result in reduced affinity for glufosinate and serve as a potential mechanism of resistance in weedy species. However, such a mutation could result in severe fitness costs as the ability of glutamate to bind to the active site would likely be reduced as well (Tankano and Dayan 2020).

Dicamba. Dicamba [3,6-dichloro-2-methoxybenzoic acid] is member of the benzoic acid chemical family and has been classified as a plant growth regulator (PGR) or synthetic auxin herbicide under the WSSA Group 4 SOA (Mallory-Smith and Retzinger 2003; Shaner 2014c). Released for commercial use in 1967, dicamba's primary use has been for POST control of broadleaf weeds in monocot species such as corn, cereal grains, and other grasses (Shaner 2014c). Dicamba can also be used in EPP or PRE applications prior to the planting of corn or dicamba-resistant soybean at a rate of 560 g ae ha^{-1}. Postemergence applications in corn can be made at a rate of 280 g ae ha^{-1}, whereas POST applications in dicamba-resistant soybean are only labeled at 560 g ae ha^{-1} (Anonymous 2020d). Applications of dicamba made EPP, PRE, or POST can provide short to moderate soil residual activity for the control of certain broadleaves (Hartzler 2017; Johnson et al. 2010).

Dicamba is a weak acid with a pKa value of 1.87, a K_{ow} of 0.29, and a K_{oc} of 2 mL g^{-1} (Shaner 2014c). These characteristics support the findings of Burnside and Lavy (1966) which demonstrated little to no adsorption of dicamba molecules to soil, increasing the risk for leaching through the soil profile into groundwater systems. However, the leaching potential of dicamba is significantly reduced by its rapid half-life of 4.4 days (Burnside and Lavy 1966). Degradation of dicamba in the soil occurs mainly by biological means with 3,6-dichlorosalicylic acid being the only major metabolite prior to mineralization into CO_2 (Krueger et al. 1991). Metabolism of dicamba is similar in both aerobic and anaerobic soils, however, the rate of metabolism in anaerobic soils is reduced compared with aerobic soils.

When used commercially as an herbicide, dicamba is formulated as a salt. Currently, four different salt formulations are commercially available including: diglycolamine (DGA), dimethylammonium, Na (sodium) salt, and N, N-Bis-(aminopropyl) methylamine (BAPMA). Dicamba salt formulations labeled for use in dicamba-tolerant soybean include the BAPMA salt, as well as the DGA salt when used with an acetic acid/acetate pH modifier (Mueller and Steckel 2019). When formulated as a salt instead of as the free acid, the solubility of dicamba in water significantly increases. The free acid of dicamba has a solubility of 4,500 mg L^{-1} whereas the dimethylamine salt of dicamba has a solubility of 720,000 mg L^{-1} (Shaner 2014c). Dicamba also has a relatively high vapor pressure of 4.5 x 10^{-3} Pa at 25º C (Shaner 2014c). With a high vapor pressure, it is possible for dicamba to evaporate off plant or soil surfaces following application, resulting in the potential for off-target movement (Hartzler 2017). Behrens and Leuschen (1979) found that dicamba was more likely to evaporate off crop leaves than it was from a silt loam soil. Although commercial formulations of dicamba have significantly lower vapor pressures than that of the free acid, dissociation of the dicamba molecule from the formulated salt can occur following application (Behrens and Leuschen 1979).

Classified as a systemic herbicide, dicamba molecules are capable of translocating throughout the plant in both the xylem and the phloem and accumulate at the growing point (Chang and Born 1971). Characteristic symptomology of dicamba exposure in susceptible plants includes the following: twisting and curling of the stems and petioles (epinasty), stem swelling and elongation, leaf cupping, overall plant stunting, and death of the growing points (Grossmann 2010; Shaner 2014c). Symptomology is first observed in the newest growth of the effected plant where metabolic activity is high (Change and Born 1971). Although the mechanism of action for dicamba is not fully understood, it is known that auxin receptors are overloaded when a susceptible plant is exposed to the herbicide, creating a rapid accumulation of ethylene, abscisic acid, and reactive oxygen species (Grossmann 2010).

As a volatile molecule, there are concerns surrounding the use of dicamba POST in soybean weed control systems. Many variables influence the volatility of dicamba following an in-crop application including herbicide formulation; herbicide rate, tank additives, nozzle type, spray droplet size, spray pressure, boom height, time of day, and various environmental conditions such as wind speed, stability of the atmosphere (inversion potential), temperature, and humidity (Carlsen et al. 2006; Ellis and Griffin. 2002). Reductions in dicamba volatility have been observed under cooler temperatures and higher relative humidity levels (Behrens and Lueschen 1979; Grover 1975). More recently, Mueller and Steckel (2019) were unable to determine any clear patterns between relative humidity and dicamba volatility. Grant and Mangan (2019) suggested that temperature inversions may be responsible for the off-target movement of dicamba in some cases. As defined by the Merriam-Webster dictionary, an inversion is “an increase of temperature with height through a layer of air” (Merriam-Webster 2020). Temperature inversions typically occur during sunset, sunrise, or under calm wind conditions (Gage et al. 2019). Fine spray particles or vapors are believed to move with wind currents or down drafts following an inversion (Ellis and Griffin 2002). Requirements of applicators have been standardized across all dicamba products used in dicamba-resistant soybean beginning 2018 and dicamba-specific trainings are required in order to purchase or apply dicamba in soybean (US-EPA 2018). Dicamba product labels set forth requirements of the applicator to minimize the risks for off-target movement. These requirements directly correlate to the variables discussed previously. Such requirements include but are not limited to: wind speeds of 5 – 16 km hr^{-1}, no application when a sensitive crop is downwind, nozzles that significantly reduce driftable fines and produce ultra-coarse or larger droplets, the use of an approved drift-reduction agent (DRA), a minimum carrier volume of 140 L ha^{-1}, and a maximum boom height of 60 cm above the targeted pest (Anonymous 2020d).

Herbicide Tank-Mixing and Interactions

The application of multiple herbicides in a single mixture has become standard practice in weed management systems. Advantages of tank-mix combinations include saving time and labor by reducing the number of trips made across a field as well as broadening the spectrum of weed control (Hatzios and Penner 1985). Applications containing two or more herbicides can be synergistic, antagonistic, or additive in nature when compared with applications of each individual product (Colby 1967; Hatzios and Penner 1985). Such responses are determined by calculating a predicted response and comparing it to an expected response of the herbicide combination (Colby 1967; Flint et al. 1988; Gowing 1960). A combination of herbicides is characterized as synergistic if the observed control value is significantly greater than the calculated expected value. Similarly, a combination of herbicides is characterized as antagonistic if the observed value is significantly less than what is predicted. If the observed and expected values are not different from each other, the combination is deemed to be additive in nature. Previous research has shown that antagonisms can occur when tank-mixing glyphosate and glufosinate, as well as glyphosate and dicamba. Tank-mix interactions have not been as clear with mixtures of glufosinate and dicamba, or the three-way mix of glyphosate, glufosinate, and dicamba. Such interactions have varied by species, use rates, and weed size.

Although previous research is limited, applications containing combinations of glyphosate and glufosinate are generally accepted to be antagonistic in nature. Bethke et al. (2013) reported glyphosate/glufosinate antagonisms both in the field and in the greenhouse across annual broadleaf and annual grass species. Synergisms with the combination of glyphosate plus glufosinate were observed in velvetleaf and common lambsquarters 7 DAT but dissipated into an additive or antagonistic response by 28 DAT depending on the rates used. The greatest antagonisms occurred when glyphosate applied at 840 g ae ha^{-1} was tank-mixed with glufosinate at 118 g ai ha^{-1}. Similarly, Meyer et al. (2020b) reported antagonistic responses in control of barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) when glufosinate was mixed with glyphosate at multiple rates. The mechanism by which this antagonism occurs has been attributed to the rapid activity of glufosinate (Bethke et al. 2013; Chuah et al. 2008; Kudsk and Mathiassen 2004). Besancon et al. (2018) identified reduced uptake and translocation of glyphosate in giant foxtail when using reduced rates of glyphosate and glufosinate were applied. Meyer et al. (2020a) reported up to a 10% reduction in absorption of radiolabeled glyphosate in barnyardgrass and Palmer amaranth when glyphosate was applied with glufosinate. Similarly, absorption of glufosinate was reduced in both species with the addition of glyphosate.

Applications of glyphosate and dicamba have also been reported to be antagonistic. Antagonistic responses from this herbicide combination have primarily been reported in monocot species. Although dicamba does not have any POST activity on these species, it appears to influence glyphosate activity and reduce control when added to a mixture. O’Sullivan and O’Donovan (1980) observed a reduction in barley (*Hordium vulgare* L.), oat (*Avena sativa* L.), and wheat control when dicamba was added to glyphosate. Flint and Barrett (1989) observed similar results with applications of glyphosate and dicamba to johnsongrass. Applications of dicamba tank-mixtures with glyphosate have also resulted in reduced barnyardgrass control (Meyer et al. 2015; Meyer et al. 2019; Meyer et al. 2020a). Meyer et al. (2015) observed reductions in barnyardgrass control when applications of glyphosate and dicamba were made on 20 cm tall plants compared with plants that were only 8 cm tall at the time of application. Weed control antagonisms have also been documented in broadleaf crops with applications of glyphosate and dicamba. Ou et al. (2018) observed reduced translocation of glyphosate and dicamba in kochia, resulting in poor control. In contrast, Spaunhorst and Bradley (2013) document an increase in control of glyphosate-resistant common waterhemp when glyphosate was added to dicamba.

Limited research has been performed examining interactions of glufosinate and dicamba tank-mixtures. Merchant et al. (2013) observed no difference in control of Texas signalgrass (*Urochloa texana* (Buckley) R. Webs.) and broadleaf signalgrass (*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webs.) when dicamba was added to applications of glufosinate whereas control of Palmer amaranth increased from 76% to 92% when glufosinate was added to dicamba applications. Likewise, Chahal and Johnson (2012) observed similar control of glyphosate-resistant horseweed with applications of dicamba plus glufosinate, compared with glufosinate applied alone. Mixtures of glufosinate and dicamba were deemed to be additive in nature for controlling giant ragweed in glufosinate-resistant corn (Ganie and Jhala 2017). Although not examined as an interaction, glyphosate-resistant common lambsquarters control was 44% when glufosinate applied alone compared with 78% when the application contained both glufosinate and dicamba (Chahal and Johnson 2012). Joseph et al. (2018) reported a synergism in sicklepod (*Senna obtusifolia* (L.) H.S. Irwin & Barneby) control when using applications of glufosinate and dicamba in the greenhouse. When used as a rescue application in XtendFlex® cotton, applications of 590 g ai ha^{-1} glufosinate plus 560 g ae ha^{-1} dicamba provided 91% control of Palmer amaranth 11 days after treatment compared with 66% control with glufosinate alone (Vann et al. 2017). Meyer et al. (2020a) observed limited metabolism of dicamba in barnyardgrass and Palmer amaranth 48 h after application when tank mixed with glufosinate.

Herbicide antagonisms are more likely to occur on larger weeds as a result of increased plant surface area and physiological processes (Burke et al. 2002; Miller et al. 2015). As a result, understanding how mixtures perform on various weed species and weed sizes is important for determining application timing. Applications containing multiple herbicides should also be made to smaller weeds to achieve optimal control. Another option for overcoming antagonistic tank-mixtures is to make sequential applications of each herbicide (Burke et al. 2002; Green 1989). Putnam and Ries (1967) reported an antagonistic response when applying amitrole and paraquat together while sequential applications of each herbicide resulted in a synergistic response.

However, sequential applications are not the answer to all herbicide applications. DeGennaro et al. (1984) reported that sequential applications of chlorimuron and quizalofop did not overcome the tank-mix antagonism that existed when controlling barnyardgrass and giant foxtail. It should also be noted that although labeled rates of herbicides may perform in an additive nature, antagonisms may still occur at the physiological level with reductions in herbicide uptake, translocation, and metabolism (Ou et al. 2018).

As we continue to build our knowledge base on how glyphosate, glufosinate, and dicamba can be used in XtendFlex® soybean, we have research questions that remain to be answered with confidence. The investigation of these questions prior to the commercialization of this technology will be beneficial to soybean growers not only in Michigan, but also across the Midwest and other areas where US soybean production occurs. Understanding how to utilize this new herbicide system will help to insure the longevity of these critical herbicides for effective weed management options in soybean.

Questions that remain to be answered:

1. What are the best strategies to manage glyphosate-resistant common waterhemp and horseweed in XtendFlex® soybean?
2. How does herbicide selection and order of application effect weed control in a two-pass POST herbicide program?
3. Are there any advantages or disadvantages to specific POST herbicide tank-mixtures?

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CHAPTER II

WEED CONTROL SYSTEMS IN CONVENTIONAL AND NO-TILL XTENDFLEX® SOYBEAN

Abstract

Glyphosate-resistant (GR) weeds continue to challenge soybean growers across the United States. XtendFlex® soybean offer farmers the flexibility to use dicamba, glyphosate, and glufosinate for weed control. Three field experiments were conducted in 2019 and 2020 to evaluate control of GR-waterhemp, GR-horseweed, and several non-GR weeds in conventional- and no-till XtendFlex® soybean. All PRE flumioxazin followed by POST treatments provided greater than 90% waterhemp control, with the exception of glyphosate POST (63%), 28 d after POST (DAPO). In a two-pass POST system, treatments that contained at least one effective herbicide, dicamba and/or glufosinate, in each application provided greater than 90% control of waterhemp. Treatments containing only one effective POST herbicide during the growing season provided 78-80% control. There were very few differences in horseweed control. Treatments that contained glufosinate POST following EPP treatments containing metribuzin + dicamba, 2,4-D, or saflufenacil resulted in greater than 99% control, 21 DAPO. Treatments that included dicamba and not glufosinate, dicamba (96%) and dicamba + glyphosate (87%), still provided acceptable control of horseweed, 21 DAPO. For non-GR weeds, the addition of dicamba to glufosinate POST reduced annual grass (barnyardgrass, giant foxtail, and yellow foxtail) control, from 98% to 89%, 14 DAPO. Common lambsquarters, Powell amaranth, and common ragweed control was greater than 90% with all treatments, 28 DAPO. The least expensive herbicide programs did not necessarily have the highest economic returns. Soybean yield and overall economic returns were significantly reduced where weeds were not controlled, demonstrating the importance for effective weed control. Overall weed control in this new soybean technology was excellent when

at least one effective herbicide was used in two separate applications. However, caution should be taken with certain tank-mixtures, since reduced control has been observed, especially on grass weeds.

Nomenclature: Dicamba; glufosinate; glyphosate; soybean, *Glycine max* (L.) Merr. GLXMA; common lambsquarters, *Chenopodium album* L. CHEAL; common ragweed, *Ambrosia artemisiifolia* L. AMBEL; horseweed, *Erigeron canadensis* L. ERICA; Powell amaranth, *Amaranthus powellii* S. Watson AMAPO; waterhemp, *Amaranthus tuberculatus* (Moq.) J.D. Sauer AMATU; barnyardgrass, *Echinochloa crus-galli* (L.) P. Beauv.; giant foxtail, *Setaria faberi* Herrm.; yellow foxtail, *Setaria pumila* (Poir.) Roem. & Schult..

Keywords: 3-way resistant soybean; antagonisms; dicamba-, glyphosate-, and glufosinate-resistant soybean; glyphosate-resistant horseweed; glyphosate-resistant waterhemp; weed control systems.

Introduction

Across the United States (U.S.), effective weed management strategies have continued to be an important component in soybean (*Glycine max* (L.) Merr.) production systems. Weedy species directly compete with soybean for light, nutrients, and water, ultimately reducing crop yield and limiting economic returns (Harre and Young 2020; Monks and Oliver 1988; Munger et al. 1987; Patterson 1995; Stoller and Woolley 1985; Zollinger and Kells 1993). Since their commercialization, glyphosate-resistant (GR) crops have provided growers with an effective, yet simple solution for broad-spectrum weed control across cropping systems. However, the overreliance on this single weed management strategy has increased the selection pressure for glyphosate-resistance in weed species and thus resulted in the development and broad distribution of glyphosate-resistant weeds (Owen 2008; Powles 2008; Young 2006). Glyphosate-resistant weeds, such as waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) and horseweed (*Erigeron canadensis* L.), continue to challenge soybean growers across the U.S. and traditional soybean technologies offer growers limited options for effective management of glyphosate-resistant weed species.

Some of the attributes that make waterhemp and horseweed both management challenges include season-long emergence, prolific seed production and innate ability to develop resistance to herbicides, including glyphosate (Bhowmik and Bekech 1993; Buhler and Owen 1997; Hager et al. 1997; Hartzler et al. 1999; Horak and Loughin 2000; Sellers et al. 2003). Germination of waterhemp occurs rapidly under optimal soil temperatures and moisture. However, germination of waterhemp has been observed throughout the growing season and has been documented to occur for up to a 70 d period (Leon and Owen 2006). Once emerged, waterhemp grows rapidly and accumulates biomass quicker than most other weed species (Hartzler et al. 2004; Horak and Loughin 2000; Sellers et al. 2003). Horak and Loughin (2000) found that waterhemp has a vertical growth rate upwards of 0.16 cm GDD^{-1} and biomass accumulation occurring at a rate of 0.30 g g^{-1} day^{-1}. Waterhemp is capable of producing 300,000 to >1,000,000 seeds per plant in the absence of competition and under optimal growing conditions (Hartzler et al. 2004; Sellers et al. 2003; Steckel et al. 2003). If waterhemp emerges at the same time as soybean, yield can be reduced upwards of 56% (Bensch et al. 2003). However, delaying waterhemp emergence beyond the V4 growth stage in soybean is likely to negate any impacts on crop yield (Hartzler et al. 2004; Steckel and Sprague 2004).

Unlike waterhemp, horseweed's relative growth rate is relatively low (Davis et al. 2009; Horak and Loughin 2000). While emergence of horseweed has been documented throughout the growing season; peak emergence typically occurs in May and in late August to early September in the North Central Region of the U.S. (Buhler and Owen 1997; Tozzi and Van Acker 2014). However, Schramski et al. (2020) reported a shift in the primary emergence pattern of horseweed in Michigan from a winter-annual pattern to a summer-annual pattern. Horseweed can produce 200,000 seeds per plant under optimal conditions and seed is dispersed by wind to upwards of 500 m that is facilitated by an attached pappus (Bhowmik and Bekech 1993; Dauer et al. 2007). Few studies have examined the effect of horseweed competition with crops. However, yield reductions up to 83% have been reported from unmanaged horseweed in soybean (Bruce and Kells 1990). The extended emergence of both waterhemp and horseweed make it difficult to achieve season-long control with preemergence (PRE) herbicides; while their growth habits make the timing of postemergence (POST) applications difficult. Across the U.S., waterhemp has been confirmed resistant to seven different herbicide sites of action and horseweed to five (Heap 2020). The development of herbicide resistance in these species, especially to glyphosate, has left growers with limited options for POST control of these weeds.

With a limited number of effective herbicide options available for waterhemp and horseweed control in traditional soybean technologies, new soybean technologies, such as XtendFlex® soybean, may provide farmers the greatest opportunity for in-season management of these weeds. This new soybean technology provides farmers the flexibility of using dicamba (WSSA Group 4), glyphosate (WSSA Group 9), and glufosinate (WSSA Group 10) for weed control, while allowing for the concurrent use of two effective herbicide sites of action to control GR weeds. Other herbicides that are used to control herbicide-susceptible and GR populations of waterhemp and horseweed include metribuzin (WSSA Group 5), and the protoporphyrinogen oxidase inhibitors (WSSA Group 14), and in the case of waterhemp the long-chain fatty acid inhibitors (WSSA Group 15) (Legleiter et al. 2009; Mueller et al. 2005). The success of the previously described chemical management strategies is a result of the susceptibility of waterhemp and horseweed to these herbicide sites of action. However, both waterhemp and horseweed are often resistant to multiple herbicide sites of action and as a result, management strategies need to be based on the use of effective herbicides (Heap 2020). Multiple applications will likely be needed to provide acceptable control and to compensate for waterhemp and horseweed's prolonged emergence patterns and growth rates. Previous research has demonstrated the ability of a dicamba- and glyphosate-based weed control system, as well as a glufosinate-based weed control system, to control glyphosate-resistant weeds independently from each other (Beyers et al. 2002; Byker et al. 2003; Coetzer et al. 2002; Eubank et al. 2008; Kruger et al. 2010; Spaunhorst and Bradley 2013; Steckel et al. 2006). Therefore, the objectives of this research were to 1) develop and evaluate various weed control systems that utilize dicamba, glyphosate, and glufosinate cooperatively for the management of glyphosate-resistant waterhemp and horseweed and other non-glyphosate resistant weeds in XtendFlex® soybean and 2) identify herbicide programs that provide the highest economic returns in XtendFlex® soybean.

Materials and Methods

Three separate field experiments were conducted in 2019 and two experiments in 2020 to evaluate control of GR waterhemp, GR horseweed, and several non-GR weeds in conventional tillage and no-tillage XtendFlex® soybean. Conventional tillage experiments were conducted at the MSU Agronomy Farm in Ingham County, MI (42.685556º N; 84.488056º W) and in Isabella County, MI (43.518333º N; 84.664444º W) in a commercial production field with a known population of GR waterhemp. The no-tillage experiments were also conducted at the MSU

Agronomy Farm. Soil characteristics for each location can be found in Table 2.1. All experiments were arranged in a randomized complete block design with four replications and plot sizes ranged from 3 m wide x 7.5 to 10 m long.

Conventional Tillage Experiments. At the Ingham County location, field preparation consisted of chisel plowing followed by one-pass of a soil finisher in the fall and two-passes of a soil finisher in the spring. Field preparation at the Isabella County location included spring disking followed by a cultimulcher. Immediately after field preparation, XtendFlex® soybean (Bayer CropScience, St. Louis, MO) was planted in 76 cm rows at seeding rates of 333,600 and 385,500 seeds ha^{-1} in 2019 and 2020, respectively. Lower seeding rates in 2019 were a result of limited seed supplies. Planting dates and soybean variety information can be found in Table 2.1.

Herbicide treatments evaluated consisted of preemergence (PRE) followed by (fb) postemergence (POST) or early-POST (EPOS) fb late-POST (LPOS) herbicide applications. All herbicides were applied using a tractor-mounted compressed air sprayer calibrated to deliver 177 L ha^{-1} at a pressure of 207 kPa. AIXR 11003 and TTI 11003 nozzles (TeeJet, Spraying Systems CO., Wheaton, IL 60187) were used for the PRE and all POST herbicide applications, respectively. Herbicide product information including use rates and application timings can be found in Table 2.2. Herbicide application dates are listed in Table 2.1 and individual treatments can be found in Tables 2.3 and 2.4 for the Ingham County and Isabella County experiments, respectively. Appropriate adjuvants were included with all herbicide treatments (Tables 2.3 and 2.4). POST and EPOS herbicide applications were made when average weed height was 10 cm. LPOS herbicide applications were made ~21 d after EPOS applications. Untreated controls were included in all studies for a comparison.

Soybean injury and weed control were evaluated weekly, beginning 21 d after planting (DAP). Weeds evaluated at the Ingham County location included: annual grasses, which included barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.), giant foxtail (*Setaria faberi* Herrm.), and yellow foxtail (*Setaria pumila* (Poir.) Roem. & Schult.); common lambsquarters (*Chenopodium album* L.); common ragweed (*Ambrosia artemisiifolia* L.); and Powell amaranth (*Amaranthus powellii* S. Watson). Weed densities at the time of POST in the untreated controls were 150/250, 54/10, 86/10, and 32/32 plants m^{-2} for annual grasses, common lambsquarters, common ragweed, and Powell amaranth in 2019/2020, respectively. GR waterhemp at 205 plants m^{-2} was the dominate weed species at the Isabella County location. Soybean injury and weed control evaluations were based on a scale of 0 to 100%, with 0 being no crop injury or weed control and 100 indicating complete plant death. Aboveground weed biomass was harvested from two 0.25 m^{-2} quadrats per plot when soybean reached maturity. Biomass was dried at 60° C for approximately 1 wk and then weighed. Percent biomass reduction was calculated using equation 1. Soybean was harvested for yield at Ingham County only using a small-plot research combine (Massey-Ferguson 8XP, AGCO, Duluth, GA). Soybean yields were adjusted to 13% moisture.

$$y = \left(100 - \left(\frac{sample\ dry\ weight}{untreated\ control\ dry\ weight}\ x\ 100\right)\right) \qquad \text{[EQ. 1]}$$

No-Tillage Experiments. Herbicide treatments evaluated consisted of early preplant (EPP) herbicide treatments applied ~14 d prior to planting with and without residual herbicides followed by EPOS or POST applications, respectively. Herbicide product information can be found in Table 2.2 and individual treatments examined are listed in Table 2.7. Herbicides were applied using the same equipment as described above through TTI 11003 nozzles (TeeJet, Spraying Systems CO., Wheaton, IL 60187). Herbicide application dates can be found in Table 2.1. Appropriate adjuvants were included with all herbicide treatments. EPOS and POST herbicide treatments were applied when average horseweed height was 10 cm. An untreated control treatment was included for comparison.

Soybean injury and weed control were evaluated weekly, beginning 7 d after EPP (DAEPP). Weeds evaluated included GR horseweed, common lambsquarters, common ragweed, and Powell amaranth. Weed densities in the untreated control were 54/32, 22/10, 10/10, and 86/10 plants m^{-2} for GR horseweed, common lambsquarters, common ragweed, and Powell amaranth in 2019/2020, respectively. Soybean injury and weed control evaluations were based on a scale of 0 to 100%, as described previously. Aboveground weed biomass was harvested from two 0.25 m^{-2} quadrats per plot at the time of soybean harvest. Biomass was separated into horseweed and non-GR weeds, dried, weighed, and percent biomass reduction was calculated. Soybean was harvested and yield was adjusted to 13% moisture.

Economic Analysis. The net economic returns in response to each herbicide program was calculated by subtracting estimated treatment costs from gross income for the Ingham County experiments. Gross income was calculated in USD ($) ha^{-1} by multiplying soybean yield by a soybean price of $0.48 kg^{-1} ($13.00 bu^{-1}). The cost of each herbicide treatment was calculated using the average herbicide and adjuvant price from January 2021 price sheets provided by major agricultural retailers in the Midwest. A custom application fee of $19.77 ha^{-1} was also included for each herbicide application timing in a program. Additional soybean production costs (seed, equipment, etc.) were not included in this analysis.

Statistical Analysis. Statistical analysis was conducted using analysis of variance (ANOVA) in R v. 3.6.0 (R Development Core Team 2019) using the lmer function. The statistical models consisted of site-year (individual year and location) and herbicide treatment as fixed effects and replication nested in site-year as the random effect. Replications were used as an error term for testing the effect of site-year, and data were combined over site-year when the interaction of site-year and herbicide treatment was not significant. Additionally, a preplanned contrast was performed to compare application systems within each experiment (PRE fb POST vs. EPOS fb LPOS or EPOS vs. POST). The assumption of normality was checked by examining histogram and normal probability plots of the residuals. Unequal variance assumption was assessed by visual inspection of side-by-side box plots of the residuals followed by the Levene's test for unequal variances. In cases of marked deviations from normality, the data were log-transformed and further analyses were performed using the transformed data. For all experiments, treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$.

Results and Discussion

Conventional Tillage Experiments. Soybean injury from flumioxazin PRE varied by location and year (data not shown). At Isabella and Ingham Counties in 2019, flumioxazin injured soybean 8 and 17%, respectively, 21 d after PRE (DAPRE). However, by the time of the POST application soybean injury was less than 5%. In 2020, soybean injury from flumioxazin was not significant. None of the postemergence herbicide programs examined resulted in significant soybean injury, with the exception of glyphosate + fomesafen + acetochlor POST (8%) at Isabella County in 2019 (data not shown).

Combined over 2019 and 2020, all PRE fb POST treatments examined provided greater than 99%, 95%, and 94% control of common lambsquarters, common ragweed, and Powell amaranth, respectively, 14 DAPO at Ingham County (Table 2.3). At the same point in time, the addition of dicamba to glufosinate POST following flumioxazin PRE reduced annual grass control from 98% to 89%, demonstrating a potential tank-mix antagonism. The addition of acetochlor to this mixture reduced annual grass control even further (82%). All other POST treatments provided >98% control of annual grass species, 14 DAPO. At the time of LPOS application, annual grass control was greatest with EPOS applications of glyphosate, glufosinate, and dicamba + glyphosate, 21 DAEP (14 DAPO) (Table 2.3). However, an EPOS application of dicamba + glufosinate only provided 79% control of annual grasses. Common lambsquarters and common ragweed control was greater with applications of dicamba + glyphosate and dicamba + glufosinate compared with either glyphosate or glufosinate applied alone, 21 DAEP. However, the addition of dicamba to EPOS applications of glyphosate or glufosinate did not change control of Powell amaranth compared with glyphosate and glufosinate applied alone.

Flumioxazin PRE controlled GR waterhemp 76% at the time of POST in Isabella County (data not shown). By 14 DAPO, waterhemp control was 67% with flumioxazin PRE followed by glyphosate POST (Table 2.4). All other PRE fb POST treatments, including glufosinate + dicamba (95%) and glufosinate + dicamba + acetochlor (95%), provided greater than 92% control of waterhemp. At the time of LPOS application, EPOS applications of glyphosate controlled waterhemp 50 to 66%, while applications of glufosinate, dicamba + glyphosate, and dicamba + glufosinate controlled waterhemp 88 to 92%, 98 to 99%, and 90%, respectively, demonstrating the importance of including effective herbicide sites of action in any postemergence application.

Control of common lambsquarters, common ragweed, and Powell amaranth was excellent 28 DAPO, or 14 DALP, at Ingham County (Table 2.5). All PRE fb POST treatments examined provided greater than 96%, 91%, and 94% control of common lambsquarters, common ragweed, and Powell amaranth, respectively. Annual grass control was greater than 92% with all POST treatments, with the exception glufosinate + dicamba (79%) and glufosinate + dicamba + acetochlor (74%) as previously mentioned. At this time, all EPOS fb LPOS herbicide programs provided greater than 98% control of annual grasses, common lambsquarters, common ragweed, and Powell amaranth. However, in Isabella County GR waterhemp control was only 35% with two applications of glyphosate (EPOS fb LPOS), 14 DALP (Table 2.4). Flumioxazin PRE followed by glyphosate POST controlled waterhemp 65% and all EPOS fb LPOS herbicide programs that contained only one effective herbicide provided 80 to 82% waterhemp control. All remaining treatments, which utilized at least one or more effective herbicide sites of action per application, provided greater than 90% control of waterhemp, regardless of the application system. By 28 DALP, control of GR waterhemp was similar with all PRE fb POST treatments with the exception of glyphosate applied alone POST. Two-pass POST (EPOS fb LPOS) systems that contained only one effective herbicide site of action, dicamba or glufosinate, either EPOS or LPOS provided 74 to 75% control of waterhemp while treatments that contained one or more effective herbicides per application provided waterhemp control similar to many of the PRE fb POST herbicide programs. While these programs collectively provided 92 to 100% control of waterhemp, systems that exclusively used two POST applications of glufosinate or dicamba plus glyphosate would increase selection pressure for glufosinate or dicamba resistance from the repeated application of a single herbicide site of action.

Overall, control of annual grasses ($p < 0.0001$), common lambsquarters ($p < 0.0001$), common ragweed ($p = 0.0003$), and Powell amaranth ($p < 0.0001$) at Ingham County, along with waterhemp control ($p = 0.0029$) at Isabella County, was greatest with PRE fb POST herbicide programs compared with EPOS fb LPOS herbicide programs, 14 DAPO. However, by 28 DAPO, EPOS fb LPOS herbicide programs provided greater control of annual grasses ($p < 0.0001$), common lambsquarters ($p = 0.0128$), common ragweed ($p = 0.0013$), and Powell amaranth ($p = 0.0097$) at Ingham County compared with PRE fb POST herbicide programs. Waterhemp control at Isabella County was still greater with PRE fb POST herbicide programs at 14 ($p = 0.0286$) and 28 DALP ($p = 0.0399$) compared with EPOS fb LPOS herbicide programs.

At the time of soybean harvest, overall weed biomass was reduced by greater than 90% with all treatments examined at Ingham County (Table 2.5). Flumioxazin PRE fb glufosinate and glufosinate + dicamba, both with and without acetochlor, POST provided the lowest reduction of weed biomass at the time of soybean harvest (91 to 93%), likely a result of reduced annual grass control. Overall reductions in weed biomass at the time of soybean harvest were greater with EPOS fb LPOS herbicide programs than with PRE fb POST herbicide programs at Ingham County ($p < 0.0001$). Similarly, at Isabella County waterhemp biomass was reduced 80 to 100% with all treatments 28 DALP (Table 2.4). Few differences in waterhemp biomass reduction arose between treatments. Eighteen out of the twenty treatments evaluated reduced biomass by 96% to 100%; the remaining two treatments reduced biomass by 80% to 90%. Flumioxazin PRE followed by glyphosate POST reduced waterhemp biomass by 90% while two applications of glyphosate POST reduced biomass by 80%. Similar to visual control ratings, reductions in waterhemp biomass 28 DALP were greater with PRE fb POST herbicide programs ($p = 0.0360$) compared with EPOS fb LPOS herbicide programs. All PRE fb effective POST treatments reduced waterhemp biomass by 99% to 100%. Advantages to the addition of acetochlor to glufosinate, dicamba plus glyphosate, dicamba plus glufosinate, or the 3-way mixture of dicamba, glyphosate, and glufosinate, POST were not evident at Ingham County or Isabella County in any site-year. This is likely a result of glyphosate's ability to effectively control the non-resistant weed species that were present in Ingham County and a result of delayed planting from excessive rainfall that shortened growing season in Isabella County.

The interaction between herbicide treatment and site-year on soybean yield was significant, so results were analyzed separately by site-year. When non-GR weed species were left unmanaged, soybean yield was reduced upwards of 66% in 2019 and 81% in 2020 (Table 2.6). Soybean yield in the untreated control were 1,386 and 828 kg ha^{-1} in 2019 and 2020, respectively. In 2019, the highest soybean yield was achieved when flumioxazin PRE was followed by glyphosate plus glufosinate POST (4,038 kg ha^{-1}). Significant reductions in yield from this top performing treatment were observed with three PRE fb POST and three EPOS fb LPOS herbicide programs. Flumioxazin PRE followed by POST applications of glufosinate, dicamba + glufosinate, or dicamba + glyphosate + glufosinate + acetochlor resulted in reduced soybean yield compared with the top yielding herbicide program. Similarly, the two-pass POST programs (EPOS fb LPOS) of glufosinate fb glyphosate, dicamba + glyphosate fb glufosinate, and dicamba + glyphosate fb dicamba + glyphosate also resulted in soybean yield reductions compared with the top yielding treatment. No differences in soybean yield between treatments were observed in 2020 with the exception of the untreated control.

Herbicide treatments examined to control non-GR weeds in conventionally tilled soybean in Ingham County ranged in cost from 77.52 to $202.77 ha^{-1} (Table 2.6). Flumioxazin PRE followed by glyphosate POST was the least expensive treatment and provided an economic return amongst the highest of the treatments evaluated. The highest economic return in 2019 was provided by flumioxazin PRE followed by glyphosate + glufosinate POST ($1,821 ha^{-1}). Economic returns were significantly reduced with eight different herbicide programs including five PRE fb POST programs and three EPOS fb LPOS programs. Applications of dicamba + glufosinate and dicamba + glufosinate + acetochlor were two of these programs that provided lower economic returns, likely a result in decreased annual grass control. Minor differences in soybean yield arose in 2020 and as a result, net income was influenced by individual treatment cost in these programs to a much greater extent. The highest economic return in 2020 was provided by glyphosate EPOS followed by glufosinate LPOS ($1,994 ha^{-1}). Four of the herbicide programs evaluated reduced economic returns compared with the highest returning treatment, including: flumioxazin PRE followed by dicamba + glufosinate, dicamba + glufosinate + acetochlor, and dicamba + glyphosate + glufosinate, as well as two POST applications of dicamba + glufosinate.

Results from this research support previous findings that both dicamba- and glufosinate-based weed control in soybean are effective in managing weeds, especially waterhemp, in conventionally tilled soybean (Beyers et al. 2002; Coetzer et al. 2002; Haas and Muller 1987; Spaunhorst and Bradley 2013; Steckel et al. 1997; Striegel et al. 2020). From this research, we conclude that both PRE fb POST and EPOS fb LPOS herbicide programs provide effective control of various non-glyphosate resistant weeds in conventional soybean. The same statement holds true when managing glyphosate-resistant waterhemp as long as an effective PRE, such as flumioxazin, is followed by a POST application that contains dicamba and/or glufosinate. In a two-pass POST system, treatments that were most effective in managing waterhemp contained dicamba and/or glufosinate in each application.

No-Tillage Experiments. None of the herbicide programs examined resulted in significant soybean injury (data not shown). Slight differences in the control of common lambsquarters, common ragweed, and Powell amaranth arose between both EPOS and POST herbicide treatments. Overall control of these weeds was excellent 28 DAEP (Table 2.7) and at the time of soybean harvest (data not shown). At 28 DAEP, control of common lambsquarters, common ragweed, and Powell amaranth was greater than 96%, 91%, and 96%, respectively, 28 DAEP. Control of common ragweed was poorest when dicamba was applied POST following a 14 EPP application of dicamba + glyphosate + metribuzin. However, an acceptable level of common ragweed control was still achieved with this treatment (91%). The remaining treatments provided greater than 97% control of common ragweed. Overall, control of common lambsquarters, common ragweed, and Powell amaranth were acceptable regardless of the herbicide treatment examined for the control of glyphosate-resistant horseweed.

Early-POST treatments that followed a 14 EPP application of glyphosate and contained glufosinate, either alone or in a mixture with dicamba and/or glyphosate, provided 93 to 100% control of horseweed 14 DAEP in 2019 (Table 2.8). When applied alone, dicamba provided 81% control of horseweed while glyphosate applied alone provided only 26% control; the combination of these two herbicides provided similar control (78%) compared with that of dicamba applied alone. In 2020, EPOS treatments of glufosinate, glyphosate + glufosinate, and dicamba + glyphosate + glufosinate provided greater than 95% control of horseweed. The addition of dicamba to glufosinate reduced control to 89% compared with glufosinate applied alone (100%). Dicamba applied alone provided 76% control of horseweed while no control was observed with an application of glyphosate. However, the combination of dicamba + glyphosate improved control to 84% compared with either herbicide applied alone. By 28 DAEP, EPOS treatments containing glufosinate still provided greater than 91 and 96% control of horseweed in 2019 and 2020, respectively. In 2019, applications of dicamba and dicamba + glyphosate only provided 85 and 83% control of horseweed, respectively, while in 2020 these same treatments provided 100% control. Glyphosate applied alone EPOS provided 65% control of horseweed 28 DAEP in 2019 while no control of horseweed was observed in 2020. Partial control of horseweed from an EPOS application of glyphosate in 2019 is likely the result of a horseweed population segregating for resistance to glyphosate.

Treatments that were evaluated EPOS following glyphosate applied 14 EPP were also evaluated POST following a 14 EPP application containing dicamba + glyphosate + metribuzin. In 2019, POST treatments containing glufosinate provided greater than 90% control of horseweed, 14 DAEP (Table 2.8). However, in 2020, all POST treatments provided greater than 90% control. By following dicamba + glyphosate + metribuzin applied 14 EPP, horseweed control from an application of dicamba increased from 81 to 91% and 76 to 93% in 2019 and 2020, respectively compared with control from a 14 EPP application of glyphosate. Similar increases in horseweed control were observed with applications of dicamba + glyphosate POST. By 28 DAEP, five of the seven POST treatments examined in 2019 provided greater than 96% control. The remaining POST treatments, glyphosate and dicamba + glyphosate, provided 85 and 87% control, respectively. In 2020, all POST treatments provided 99 to 100% control of horseweed, 28 DAEP.

To compare the effectiveness of dicamba + glyphosate + metribuzin applied 14 EPP, three additional commercially used 14 EPP treatments were examined and received glufosinate POST for comparison. These treatments included 2,4-D + glyphosate + metribuzin, saflufenacil + glyphosate + metribuzin, and dicamba + glyphosate plus a premix containing flumioxazin, metribuzin, and pyroxasulfone. Minor differences in horseweed control arose 14 DAEP in both 2019 and 2020 however horseweed control was still greater than 90%. By 28 DAEP, there were no differences in horseweed control (99 to 100%) amongst these different 14 EPP programs. At the time of soybean harvest, all treatments reduced horseweed biomass by greater than 97% combined over 2019 and 2020 with the exception of glyphosate 14 EPP followed by glyphosate EPOS (3%).

The interaction between herbicide treatment and site-year on soybean yield was not significant for no-till studies thus soybean yield was combined over site-years. Soybean yield was reduced upwards of 49% in the untreated control; soybean yield was 1,871 kg ha^{-1} (Table 2.9). The greatest observed soybean yield was with glyphosate + glufosinate POST following a 14 EPP application of dicamba + glyphosate + metribuzin (3,665 kg ha^{-1}). Dicamba applied alone both EPOS or POST resulted in significant soybean yield reductions compared with the top yielding treatment; this is likely the result of increased competition from annual grass species. However, when dicamba was combined with glyphosate either EPOS or POST, soybean yield improved compared with dicamba applied alone. Reductions in soybean yield were also observed when dicamba + glufosinate was applied POST. There were no differences in soybean yield between treatments that received glufosinate POST, regardless of the 14 EPP treatment examined.

Treatment costs for managing GR horseweed ranged from \$70.08 to \$220.81 ha^{-1} (Table 2.9). The highest economic return was provided by glyphosate (14 EPP) followed by dicamba + glyphosate EPOS (\$1,611 ha^{-1}). However, many other treatments provided an economic return that was similar to this herbicide program. Similar to the trends observed in yield, economic returns were significantly reduced with dicamba + glufosinate applied POST as well as dicamba applied both EPOS and POST compared with the highest returning treatment. The addition of glyphosate to treatments of dicamba EPOS or POST resulted in significant increases in economic return compared with when dicamba was applied alone. Economic returns were similar among 14 EPP treatments that were included for commercial comparisons.

Results from this research support previous findings around the use of glufosinate and dicamba + glyphosate in no-till soybean to control both glyphosate-resistant horseweed and non-glyphosate resistant weed species (Byker et al. 2013; Eubank et al. 2008; Haas and Muller 1987; Johnson et al. 2010; Kruger et al. 2010; Steckel et al. 1997; Steckel et al. 2006). Overall, very few differences in horseweed control arose with the herbicide treatments that were examined, especially in 2020. Many of the EPOS herbicide treatments examined provided similar control of horseweed when compared with POST treatments that followed a 14 EPP application of dicamba + glyphosate + metribuzin. This is likely a result of horseweed emergence patterns in Michigan, where peak emergence has been reported to occur towards the end of May and then continues throughout the growing season (Schramski et al. 2020). However, due to the extended emergence window of horseweed, the use of a 14 EPP application of glyphosate followed by a one-pass EPOS program may not be the most consistent long-term management strategy, especially where horseweed pressure is high. If an EPOS program fails, limited rescue options exist. For this reason, our recommendation for managing glyphosate-resistant horseweed in no-till soybean is to start clean by applying an EPP treatment that controls emerged horseweed with herbicides like dicamba, 2,4-D, saflufenacil, glufosinate, or paraquat. This EPP applications should also contain metribuzin prior to soybean planting to reduce early-season horseweed pressure. This EPP application should then be followed with an effective POST herbicide treatment, containing either dicamba and/or glufosinate, in-season to control any weeds that may have emerged. All herbicide programs examined provided excellent control of other non-glyphosate resistant weeds thus farmers should focus on managing horseweed when deciding on their POST herbicide program.

In a conventionally tilled XtendFlex® soybean system, farmers should base their herbicide programs around the weed species that are present in their fields. Two-pass POST programs show advantages in controlling non-glyphosate resistant weed species, while PRE fb POST programs are advantageous for managing glyphosate-resistant waterhemp. It should be noted that more expensive herbicide programs may offer similar or improved control of certain weed species but may not improve the overall economic return. Regardless of the strategy used, at least one effective herbicide, such as dicamba or glufosinate, should be used per postemergence application to obtain optimal control of various weed species in conventional tillage soybean. However, when allowed the use of two effective foliar sites of action POST, such as dicamba + glufosinate, will reduce selection pressure on limited effective herbicide sites of action.

Overall, the XtendFlex® soybean system give growers the flexibility to apply dicamba, glyphosate, and/or glufosinate to appropriately manage weeds in their fields. Both dicamba and glufosinate were effective in managing glyphosate-resistant weeds, such as horseweed and waterhemp. One benefit to applications containing dicamba, whether applied preemergence or postemergence, is that dicamba can provide short-term soil residual control for managing summer annual weeds (Johnson et al. 2010; Shaner 2014). This characteristic of dicamba can increase the window of weed control provided by this herbicide. However, restrictions on the dicamba label can limit its application window. Conditions that may limit that application opportunity for dicamba include: the presence of nearby sensitive crops, the presence of certain endangered species within a county, or as a result of inadequate weather conditions, such as high winds and potential temperature inversions (Anonymous 2020a). In such instances, farmers would still have the opportunity to apply glufosinate, which has been proven effective in controlling both glyphosate-resistant and -susceptible weeds. However, weed control provided by glufosinate is highly dependent on optimal weather and application conditions such as optimal sunlight, warm temperatures, high humidity, and excellent spray coverage (Anonymous 2020b). Although not examined in these studies, farmers should be aware of the potential for tank-mix antagonisms when using dicamba. In this research, we observed significant reductions in control of annual grass species when dicamba + glufosinate was applied postemergence. Previous research has also demonstrated antagonistic responses in grass control with applications of dicamba + glyphosate (Flint and Barrett 1989; Meyer et al. 2015; Meyer et al. 2019; O'Sullivan and O'Donovan 1980) and when WSSA Group 1 herbicides, like clethodim or quizalofop, are tank-mixed with dicamba (Blackshaw et al. 2006; Harre et al. 2020; Underwood et al. 2016).

APPENDIX

APPENDIX

CHAPTER II TABLES

Table 2.1. XtendFlex® soybean varieties, planting dates, herbicide application dates, and soil information for conventional and no-tillage experiments conducted in Ingham and Isabella Counties, MI (2019-2020).

	Conventional Tillage			No-tillage	
	Ingham County		Isabella County	Ingham County	
	2019	2020	2019	2019	2020
Soybean variety[a]	AG26XF0	AG22XF1	AG26XF0	AG26XF0	AG22XF1
Planting date	May 28	May 23	July 8	May 27	May 23
Application date[b]					
14 EPP	—	—	—	May 14	May 12
PRE	May 28	May 23	July 8	May 27	May 23
EPOS	June 26	June 15	July 30	July 9	June 25
POST	July 1	June 22	August 9	July 17	July 7
LPOS	July 17	July 7	August 19	—	—
Soil type	loam	loam	Sandy loam	loam	loam
Soil pH	5.7	5.7	6.1	5.7	5.7
Organic matter (%)	3.0	3.0	2.4	3.0	3.0

[a] Asgrow, Bayer CropScience, St. Louis, MO.

[b] Abbreviations: 14 EPP = 14 d early preplant; PRE = preemergence; EPOS = early postemergence; POST = postemergence; and LPOS = late postemergence.

Table 2.2. Herbicide product, application rates and timings, and manufacturer information for soybean treatments used in conventional and no-tillage XtendFlex® soybean experiments conducted in Ingham and Isabella Counties, MI (2019-2020).

Trade name	Active ingredients	Rates	Timings[a]	Manufacturer[b]
		kg ai or ae ha^{-1}		
Dimetric EXT	metribuzin	0.32	14 EPP	WinField United
Fierce MTZ	flumioxazin + metribuzin + pyroxasulfone	0.07 + 0.21 + 0.09	14 EPP	Valent U.S.A. LLC
Liberty 280SL	glufosinate	0.65	EPOS, POST, LPOS	BASF Corporation
Roundup PowerMax	glyphosate	1.26	14 EPP, EPOS, POST, LPOS	Bayer CropScience
Sharpen	saflufenacil	0.025	14 EPP	BASF Corporation
Shredder 2,4-D LV4	2,4-D ester	0.56	14 EPP	WinField United
Valor SX	flumioxazin	0.07	PRE	Valent U.S.A. LLC
Warrant	acetochlor	1.26	POST	Bayer CropScience
Warrant Ultra	acetochlor + fomesafen	1.23 + 0.28	POST	Bayer CropScience
XtendiMax	dicamba	0.56	14 EPP, EPOS, POST, LPOS	Bayer CropScience

[a] Abbreviations: 14 EPP = 14 d early preplant; PRE = preemergence; EPOS = early postemergence; POST = postemergence; and LPOS = late postemergence.

[b] Manufacturer information: WinField United, Arden Hills, MN, www.winfieldunited.com; Valent U.S.A. LLC, San Ramon, CA, www.valent.com; BASF Corporation, Research Triangle Park, NC, www.basf.com; Bayer CropScience, Research Triangle Park, NC, www.cropscience.bayer.com.

Table 2.3. Control of non-glyphosate resistant weeds 14 d after POST/ 21 d after EPOS applications in conventional tillage XtendFlex® soybean in Ingham County, MI, 2019 and 2020. Data are combined over years.

Treatment[b]	Timing		Weed control (14 DAPO/21 DAEP)[a]			
			Annual grass[c]	Common lambsquarters	Common ragweed	Powell amaranth
			% control			
Flumioxazin fb glyphosate + AMS	PRE	POST	100 a[d]	99 a	100 a	100 a
Flumioxazin fb glufosinate + AMS	PRE	POST	98 ab	99 a	96 ab	98 ab
Flumioxazin fb glyphosate + glufosinate + AMS	PRE	POST	100 a	100 a	99 a	100 a
Flumioxazin fb dicamba + glyphosate + CAR + DRA	PRE	POST	100 a	100 a	100 a	100 a
Flumioxazin fb dicamba + glufosinate + MON	PRE	POST	89 c	99 a	95 a-c	94 bc
Flumioxazin fb dicamba + glyphosate + glufosinate + MON	PRE	POST	100 a	100 a	99 a	98 ab
Flumioxazin fb glufosinate + acetochlor + AMS	PRE	POST	98 ab	99 a	96 ab	100 a
Flumioxazin fb dicamba + glyphosate + acetochlor + CAR + DRA	PRE	POST	100 a	100 a	100 a	100 a
Flumioxazin fb dicamba + glufosinate + acetochlor + MON	PRE	POST	82 d	100 a	99 a	98 ab
Flumioxazin fb dicamba + glyphosate + glufosinate + acetochlor + MON	PRE	POST	99 a	100 a	99 a	98 ab

Table 2.3. (cont'd)

Treatment	Timing	Weed control (14 DAPO/21 DAEP) Annual grasses	Common lambsquarters	Common ragweed	Powell amaranth
		——— % control ———			
Glyphosate + AMS	EPOS	95 a-c	93 b	86 d	95 a-c
Glufosinate + AMS	EPOS	91 bc	90 bc	93 bc	86 d
Glufosinate + AMS	EPOS	93 a-c	89 c	93 bc	86 d
Glufosinate + AMS	EPOS	87 cd	90 bc	90 cd	91 cd
Dicamba + glyphosate + CAR + DRA	EPOS	96 a-c	99 a	99 a	97 ab
Dicamba + glyphosate + CAR + DRA	EPOS	96 a-c	99 a	99 a	98 ab
Dicamba + glufosinate + MON	EPOS	79 d	97 a	100 a	91 cd

[a] Abbreviations: PRE = preemergence; POST = postemergence; EPOS = early postemergence; LPOS = late postemergence; 14 DAPO = 14 d after POST and 21 d after EPOS application timing.
[b] See Table 2.2 for herbicide product information and rates. Adjuvant information: AMS = ammonium sulfate at 2% w w^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, Research Triangle Park, NC).
[c] Annual grass species included: barnyardgrass, giant foxtail, and yellow foxtail.
[d] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 2.4. Control of glyphosate-resistant waterhemp at the LPOS application timing (14 DAPO), 14 d after LPOS/ 28 d after POST, and 28 d after LPOS / 42 d after POST applications in conventional tillage XtendFlex® soybean in Isabella County, MI in 2019.

Treatment[b]	Timing[c]		Waterhemp control			Biomass[a]
			14 DAPO	14 DALP	28 DALP	28 DALP
			% control			% reduction
Flumioxazin fb glyphosate + AMS	PRE	POST	67 c[d]	65 d	60 d	90 b
Flumioxazin fb glufosinate + AMS	PRE	POST	100 a	99 a	98 a	99 a
Flumioxazin fb glyphosate + glufosinate + AMS	PRE	POST	98 ab	95 a	87 a-c	99 a
Flumioxazin fb dicamba + glyphosate + CAR + DRA	PRE	POST	99 a	99 a	100 a	100 a
Flumioxazin fb dicamba + glufosinate + MON	PRE	POST	95 ab	93 ab	94 a	99 a
Flumioxazin fb dicamba + glyphosate + glufosinate + MON	PRE	POST	97 ab	99 a	99 a	99 a
Flumioxazin fb glufosinate + acetochlor + AMS	PRE	POST	99 a	96 a	95 a	99 a
Flumioxazin fb dicamba + glyphosate + acetochlor + CAR + DRA	PRE	POST	98 ab	100 a	100 a	100 a
Flumioxazin fb dicamba + glufosinate + acetochlor + MON	PRE	POST	95 ab	94 a	97 a	99 a
Flumioxazin fb dicamba + glyphosate + glufosinate + acetochlor + MON	PRE	POST	95 ab	95 a	95 a	99 a
Flumioxazin fb glyphosate + fomesafen + acetochlor + AMS + COC	PRE	POST	92 ab	90 a-c	88 ab	99 a

Table 2.4 (cont'd)

Treatment	Timing		Waterhemp control 14 DAPO	14 DALP	28 DALP	Biomass 28 DALP
			——— % control ———			% reduction
Glyphosate + AMS fb glyphosate + AMS	EPOS	LPOS	50 d	35 e	31 e	80 c
Glyphosate + AMS fb glufosinate + AMS	EPOS	LPOS	66 c	80 c	75 bc	98 a
Glufosinate + AMS fb glyphosate + AMS	EPOS	LPOS	88 b	82 c	74 c	96 a
Glufosinate + AMS fb glufosinate + AMS	EPOS	LPOS	88 b	90 a-c	92 a	99 a
Glufosinate + AMS fb dicamba + glyphosate + CAR + DRA	EPOS	LPOS	92 ab	95 a	98 a	99 a
Dicamba + glyphosate + CAR + DRA fb glufosinate + AMS	EPOS	LPOS	99 ab	100 a	100 a	100 a
Dicamba + glyphosate + CAR + DRA fb dicamba + glyphosate + CAR + DRA	EPOS	LPOS	98 ab	100 a	100 a	100 a
Dicamba + glufosinate + MON fb dicamba + glufosinate + MON	EPOS	LPOS	90 ab	92 a-c	96 a	99 a

[a] Waterhemp biomass reduction was calculated as y = (100 – ((sample dry weight / non-treated control dry weight) * 100)).
[b] See Table 2.2 for herbicide product information and rates. Adjuvant information: AMS = ammonium sulfate at 2% w w^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); COC = crop oil concentrate at 1% v v^{-1} (Herbimax, Loveland Products Inc., Loveland, CO); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, Research Triangle Park, NC).
[c] Abbreviations: PRE = preemergence; POST = postemergence; EPOS = early postemergence; LPOS = late postemergence; 14 DAPO = 14 d after POST and 21 d after EPOS; 14 DALP = 14 d after LPOS and 28 d after POST; 28 DALP = 28 d after LPOS and 42 d after POST.
[d] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 2.5. Control of non-glyphosate resistant weeds 28 d after POST/ 35 d after EPOS applications in conventional tillage XtendFlex® soybean in Ingham County, MI, 2019 and 2020. Data are combined over years.

		Weed control (28 DAPO/35 DAEP)[a]				At harvest
Treatment[b]	Timing	Annual grasses[c]	Common lambsquarters	Common ragweed	Powell amaranth	Biomass
		% control				% reduction
Flumioxazin fb glyphosate + AMS	PRE POST	97 a[d]	100 a	100 a	99 ab	99 a
Flumioxazin fb glufosinate + AMS	PRE POST	92 a	96 d	91 c	94 c	92 b
Flumioxazin fb glyphosate + glufosinate + AMS	PRE POST	96 a	99 ab	97 a	99 ab	98 a
Flumioxazin fb dicamba + glyphosate + CAR + DRA	PRE POST	95 a	100 a	100 a	100 a	98 a
Flumioxazin fb dicamba + glufosinate + MON	PRE POST	79 b	98 b-d	97 ab	94 bc	93 b
Flumioxazin fb dicamba + glyphosate + glufosinate + MON	PRE POST	95 a	100 a	99 a	97 a-c	99 a
Flumioxazin fb glufosinate + acetochlor + AMS	PRE POST	92 a	97 cd	93 bc	96 a-c	93 b
Flumioxazin fb dicamba + glyphosate + acetochlor + CAR + DRA	PRE POST	98 a	100 a	100 a	100 a	100 a
Flumioxazin fb dicamba + glufosinate + acetochlor + MON	PRE POST	74 b	99 a-c	96 ab	99 ab	91 b
Flumioxazin fb dicamba + glyphosate + glufosinate + acetochlor + MON	PRE POST	98 a	100 a	99 a	99 ab	99 a

Table 2.5. (cont'd)

Treatment	Timing	Weed control (28 DAPO/35 DAEP) Annual grasses	Common lambsquarters	Common ragweed	Powell amaranth	At harvest Biomass
		% control				% reduction
Glyphosate + AMS fb glufosinate + AMS	EPOS LPOS	98 a	100 a	98 a	100 a	99 a
Glufosinate + AMS fb glyphosate + AMS	EPOS LPOS	100 a	100 a	100 a	100 a	100 a
Glufosinate + AMS fb glufosinate + AMS	EPOS LPOS	100 a	100 a	100 a	100 a	100 a
Glufosinate + AMS fb dicamba + glyphosate + CAR + DRA	EPOS LPOS	100 a	100 a	100 a	100 a	100 a
Dicamba + glyphosate + CAR + DRA fb glufosinate + AMS	EPOS LPOS	100 a	100 a	100 a	100 a	100 a
Dicamba + glyphosate + CAR + DRA fb dicamba + glyphosate + CAR + DRA	EPOS LPOS	100 a	100 a	100 a	100 a	100 a
Dicamba + glufosinate + MON fb dicamba + glufosinate + MON	EPOS LPOS	98 a	100 a	100 a	100 a	99 a

[a] Abbreviations: PRE = preemergence; POST = postemergence; EPOS = early postemergence; LPOS = late postemergence; 28 DAPO = 28 d after POST, 14 d after LPOS.
[b] See Table 2.2 for herbicide product information and rates. Adjuvant information: AMS = ammonium sulfate at 2% w w^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, Research Triangle Park, NC).
[c] Annual grass species included: barnyardgrass, giant foxtail, and yellow foxtail.
[d] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 2.6. Herbicide treatment cost, soybean yield, and net return for herbicide programs used to manage non-glyphosate resistant weeds in conventional tillage XtendFlex® soybean in Ingham county, MI in 2019 and 2020.

Treatment[a]	Timing[b]	Treatment cost[c]	2019 Yield	2019 Net return[d]	2020 Yield	2020 Net return
		USD $ ha^{-1}	kg ha^{-1}	USD $ ha^{-1}	kg ha^{-1}	USD $ ha^{-1}
Flumioxazin fb glyphosate + AMS	PRE POST	$77.52	3,829 ab[e]	$1,752 ab	4,169 a	$1,913 a-c
Flumioxazin fb glufosinate + AMS	PRE POST	$92.15	3,011 bc	$1,347 b-e	4,260 a	$1,942 ab
Flumioxazin fb glyphosate + glufosinate + AMS	PRE POST	$106.60	4,038 a	$1,821 a	4,287 a	$1,942 ab
Flumioxazin fb dicamba + glyphosate + CAR + DRA	PRE POST	$128.54	3,647 a-c	$1,614 a-d	4,234 a	$1,893 a-d
Flumioxazin fb dicamba + glufosinate + MON	PRE POST	$143.86	3,065 bc	$1,320 c-e	3,976 a	$1,754 b-d
Flumioxazin fb dicamba + glyphosate + glufosinate + MON	PRE POST	$158.32	3,207 a-c	$1,374 b-e	3,998 a	$1,752 b-d
Flumioxazin fb glufosinate + acetochlor + AMS	PRE POST	$126.39	3,802 ab	$1,690 a-d	4,362 a	$1,957 ab
Flumioxazin fb dicamba + glyphosate + acetochlor + CAR + DRA	PRE POST	$162.79	3,810 ab	$1,658 a-d	4,257 a	$1,871 a-d
Flumioxazin fb dicamba + glufosinate + acetochlor + MON	PRE POST	$178.11	3,293 a-c	$1,394 b-e	3,967 a	$1,717 cd
Flumioxazin fb dicamba + glyphosate + glufosinate + acetochlor + MON	PRE POST	$192.57	2,859 c	$1,174 e	4,137 a	$1,784 a-d

Table 2.6. (cont'd)

Treatment	Timing	Treatment cost	2019 Yield	2019 Net return	2020 Yield	2020 Net return
		USD $ ha^{-1}	kg ha^{-1}	USD $ ha^{-1}	kg ha^{-1}	USD $ ha^{-1}
Glyphosate + AMS fb glufosinate + AMS	EPOS LPOS	$84.71	3,705 a-c	$1,685 a-d	4,350 a	$1,994 a
Glufosinate + AMS fb glyphosate + AMS	EPOS LPOS	$84.71	3,081 bc	$1,386 b-e	4,327 a	$1,982 a
Glufosinate + AMS fb glufosinate + AMS	EPOS LPOS	$99.34	3,841 ab	$1,735 a-c	4,228 a	$1,920 a-c
Glufosinate + AMS fb dicamba + glyphosate + CAR + DRA	EPOS LPOS	$135.73	3,721 a-c	$1,641 a-d	4,079 a	$1,814 a-d
Dicamba + glyphosate + CAR + DRA fb glufosinate + AMS	EPOS LPOS	$135.73	2,968 bc	$1,282 de	4,296 a	$1,915 a-c
Dicamba + glyphosate + CAR + DRA fb dicamba + glyphosate + CAR + DRA	EPOS LPOS	$172.13	3,013 bc	$1,268 de	4,136 a	$1,803 a-d
Dicamba + glufosinate + MON fb dicamba + glufosinate + MON	EPOS LPOS	$202.77	3,484 a-c	$1,460 a-e	3,941 a	$1,680 d
Untreated		$0.00	1,386 d	$662 f	828 b	$395 e

[a] See Table 2.2 for herbicide product information and rates. Adjuvant information: AMS = ammonium sulfate at 2% w w^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, Research Triangle Park, NC).

[b] Abbreviations: PRE = preemergence; POST = postemergence; EPOS = early postemergence; LPOS = late postemergence.

[c] Total treatment cost = herbicide costs + adjuvant costs + application costs. Average price of herbicides and adjuvants were calculated from multiple price lists. Application cost = $8.00 per application.

[d] Net return = (yield x price) – treatment cost. Crop selling price = $13.00 bu^{-1} (January 2021).

[e] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 2.7. Control of non-glyphosate resistant weeds 28 d after EPOS / 21 d after POST applications in no-tillage XtendFlex® soybean in Ingham County, MI, 2019 and 2020. Data are combined over years.

Treatments[a]			Weed Control (28 DAEP/21 DAPO)[b]		
14 d EPP		EPOS/POST[c]	Common lambsquarters	Common ragweed	Powell amaranth
			% control		
Glyphosate + AMS	fb	Glyphosate + AMS	99 ab[d]	99 a	100 a
	fb	Glufosinate + AMS	97 cd	98 a	99 a
	fb	Dicamba + CAR+ DRA	100 a	100 a	100 a
	fb	Glyphosate + glufosinate + AMS	97 cd	97 a	99 a
	fb	Dicamba + glyphosate + CAR + DRA	99 ab	100 a	100 a
	fb	Dicamba + glufosinate + MON	99 ab	100 a	100 a
	fb	Dicamba + glyphosate + glufosinate + MON	100 a	100 a	100 a
Dicamba + glyphosate + metribuzin + CAR + DRA	fb	Glyphosate + AMS	100 a	100 a	100 a
	fb	Glufosinate + AMS	99 ab	98 a	100 a
	fb	Dicamba + CAR + DRA	97 b-d	91 b	96 b
	fb	Glyphosate + glufosinate + AMS	98 a-d	100 a	100 a
	fb	Dicamba + glyphosate + CAR + DRA	100 a	100 a	100 a
	fb	Dicamba + glufosinate + MON	100 a	100 a	99 a
	fb	Dicamba + glyphosate + glufosinate + MON	100 a	100 a	100 a

Table 2.7. (cont'd)

Treatments			Weed Control (28 DAEP/21 DAPO)		
14 d EPP		EPOS/POST	Common lambsquarters	Common ragweed	Powell amaranth
			——— % control ———		
Saflufenacil + glyphosate + metribuzin + AMS + MSO	fb	Glufosinate + AMS	99 ab	100 a	100 a
2,4-D + glyphosate + metribuzin + AMS + MSO	fb	Glufosinate + AMS	98 a-c	100 a	99 a
Dicamba + glyphosate + flumioxazin + metribuzin + pyroxasulfone + CAR + DRA	fb	Glufosinate + AMS	96 d	99 a	100 a

[a] See Table 2.2 for herbicide product information and rates. Adjuvant information: AMS = ammonium sulfate at 2% v v^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, Research Triangle Park, NC); MSO = methylated seed oil at 1% v v^{-1} (SuperSpread, Wilbur-Ellis Co., San Francisco, CA).

[b] Abbreviations: 14 EPP = 14 d early preplant; EPOS = early postemergence; POST = postemergence; 28 DAEP = 28 d after EPOS; 21 DAPO = 21 d after POST.

[c] Postemergence applications were either applied early-POST or POST. Treatments follow a 14 EPP of glyphosate alone were applied EPOS and all other treatments were applied POST.

[d] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 2.8. Control of glyphosate-resistant horseweed 14 d after EPOS/ 7 d after POST, 28 d after EPOS/ 21 d after POST, and horseweed biomass reduction at soybean harvest in no-tillage XtendFlex® soybean in Ingham County, MI, 2019 and 2020. Data are combined over years.

Treatments[b]			Horseweed control				Biomass[a]
			14 DAEP/7 DAPO[c]		28 DAEP/21 DAPO		At harvest
14 d EPP		EPOS/POST[d]	2019	2020	2019	2020	2019 & 2020
			— % control —		— % control —		% reduction
Glyphosate + AMS	fb	Glyphosate + AMS	26 e[e]	0 g	65 d	0 d	3 b
	fb	Glufosinate + AMS	99 a	100 a	97 a	96 c	99 a
	fb	Dicamba + CAR+ DRA	81 d	76 f	85 c	100 a	99 a
	fb	Glyphosate + glufosinate + AMS	93 ab	98 a-c	91 a-c	98 b	98 a
	fb	Dicamba + glyphosate + CAR + DRA	78 d	84 e	83 c	100 a	100 a
	fb	Dicamba + glufosinate + MON	96 ab	89 de	96 ab	100 a	100 a
	fb	Dicamba + glyphosate + glufosinate + MON	100 a	95 a-d	99 a	100 a	100 a
Dicamba + glyphosate + metribuzin + CAR + DRA	fb	Glyphosate + AMS	78 d	91 cd	85 c	99 ab	97 a
	fb	Glufosinate + AMS	95 ab	99 ab	99 a	100 a	100 a
	fb	Dicamba + CAR + DRA	91 bc	93 b-d	96 ab	100 a	100 a
	fb	Glyphosate + glufosinate + AMS	100 a	90 de	100 a	100 a	100 a
	fb	Dicamba + glyphosate + CAR + DRA	85 cd	91 c-e	87 bc	100 a	100 a
	fb	Dicamba + glufosinate + MON	100 a	92 b-d	100 a	100 a	100 a
	fb	Dicamba + glyphosate + glufosinate + MON	98 ab	94 a-d	100 a	100 a	100 a

Table 2.8. (cont'd)

Treatments			Horseweed control				Biomass
			14 DAEP/7 DAPO		28 DAEP/21 DAPO		At harvest
14 d EPP		EPOS/POST	2019	2020	2019	2020	2019 & 2020
			——— % control ———		——— % control ———		% reduction
Saflufenacil + glyphosate + metribuzin + AMS + MSO	fb	Glufosinate + AMS	98 ab	91 cd	100 a	100 a	100 a
2,4-D + glyphosate + metribuzin + AMS + MSO	fb	Glufosinate + AMS	91 bc	90 de	100 a	100 a	100 a
Dicamba + glyphosate + flumioxazin + metribuzin + pyroxasulfone + CAR + DRA	fb	Glufosinate + AMS	96 ab	99 ab	100 a	100 a	100 a

[a] Horseweed biomass reduction was calculated as y = (100 – ((sample dry weight / non-treated control dry weight) * 100)).
[b] See Table 2.2 for herbicide product information and rates. Adjuvant information: AMS = ammonium sulfate at 2% v v^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, Research Triangle Park, NC); MSO = methylated seed oil at 1% v v^{-1} (SuperSpread, Wilbur-Ellis Co., San Francisco, CA).
[c] Abbreviations: 14 EPP = 14 d early pre-plant; EPOS = early postemergence; POST = postemergence; 14 DAEP = 14 d after EPOS; 7 DAPO = 7 d after POST; 28 DAEP = 28 d after EPOS; 21 DAPO = 21 d after POST.
[d] Postemergence applications were either applied early-POST or POST. Treatments follow a 14 EPP of glyphosate alone were applied EPOS and all other treatments were applied POST.
[e] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 2.9. Herbicide treatment cost, soybean yield, and net return for herbicide programs used to manage glyphosate-resistant horseweed in no-tillage XtendFlex® soybean in Ingham county, MI in 2019 and 2020. Data are combined over years.

Treatments[a]			Treatment cost[b]	Yield	Net return[c]
14 d EPP[d]		EPOS/POST[e]			
			USD $ ha^{-1}	kg ha^{-1}	USD $ ha^{-1}
Glyphosate + AMS	fb	Glyphosate + AMS	$70.08	3,219 a-d[f]	$1,468 ab
	fb	Glufosinate + AMS	$84.71	3,263 a-d	$1,473 ab
	fb	Dicamba + CAR+ DRA	$106.65	2,849 cd	$1,255 bc
	fb	Glyphosate + glufosinate + AMS	$99.16	3,450 a-c	$1,549 a
	fb	Dicamba + glyphosate + CAR + DRA	$121.11	3,627 ab	$1,611 a
	fb	Dicamba + glufosinate + MON	$136.43	3,422 a-c	$1,497 ab
	fb	Dicamba + glyphosate + glufosinate + MON	$150.88	3,640 a	$1,589 a
Dicamba + glyphosate + metribuzin + CAR + DRA	fb	Glyphosate + AMS	$140.08	3,559 ab	$1,559 a
	fb	Glufosinate + AMS	$154.71	3,506 ab	$1,520 ab
	fb	Dicamba + CAR + DRA	$176.66	2,656 d	$1,092 cd
	fb	Glyphosate + glufosinate + AMS	$169.17	3,665 a	$1,581 a
	fb	Dicamba + glyphosate + CAR + DRA	$191.11	3,472 ab	$1,468 ab
	fb	Dicamba + glufosinate + MON	$206.43	3,025 b-d	$1,238 bc
	fb	Dicamba + glyphosate + glufosinate + MON	$220.81	3,534 ab	$1,468 ab
Saflufenacil + glyphosate + metribuzin + AMS + MSO	fb	Glufosinate + AMS	$133.21	3,435 a-c	$1,507 ab
2,4-D + glyphosate + metribuzin + AMS + MSO	fb	Glufosinate + AMS	$123.48	3,522 ab	$1,559 a

Table 2.9. (cont'd)

Treatments			Treatment cost	Yield		Net return	
14 d EPP		EPOS/POST					
			USD $ ha^{-1}	kg ha^{-1}		USD $ ha^{-1}	
Dicamba + glyphosate + flumioxazin + metribuzin + pyroxasulfone + CAR + DRA	fb	Glufosinate + AMS	$204.11	3,399	a-c	$1,418	ab
Untreated			$0.00	1,871	e	$895	d

[a] See Table 2.2 for herbicide product information and rates. Adjuvant information: AMS = ammonium sulfate at 2% v v^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, Research Triangle Park, NC); MSO = methylated seed oil at 1% v v^{-1} (SuperSpread, Wilbur-Ellis Co., San Francisco, CA).

[b] Total treatment cost = herbicide costs + adjuvant costs + application costs. Treatment cost = average of price lists for herbicides and adjuvants; Application cost = $8.00 per application.

[c] Crop selling price = $0.48 kg^{-1} (January 2021). Net return = (yield x price) – treatment cost.

[d] Abbreviations: 14 EPP = 14 d early pre-plant; EPOS = early postemergence; POST = postemergence.

[e] Postemergence applications were either applied early-POST or POST. Treatments follow a 14 EPP of glyphosate alone were applied EPOS and all other treatments were applied POST.

[f] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

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CHAPTER III

INTERACTIONS OF DICAMBA, GLYPHOSATE, AND GLUFOSINATE AS TANK-MIX PARTNERS

Abstract

XtendFlex® soybean is a new trait platform which confers resistance to three herbicide sites of action that include the active ingredients dicamba, glyphosate, and glufosinate. The objective of this research was to identify potential antagonistic or synergistic weed responses from herbicide tank-mixtures used in this system. Greenhouse experiments were conducted at Michigan State University (MSU) in East Lansing, MI, to characterize the response of various glyphosate-resistant (GR) and -susceptible weed populations to tank-mixtures containing glyphosate, glufosinate, and/or dicamba. Three field experiments were also conducted in 2019 and 2020 to evaluate responses of these herbicide combinations. Results from the greenhouse experiments and the field experiments differed. Combinations of glyphosate + glufosinate were often antagonistic, especially in controlling broadleaf weed species. In the greenhouse, antagonisms were often observed with combinations of dicamba + glyphosate. However, this combination acted in either an additive or synergistic manner in the field, especially in controlling GR weeds like waterhemp and horseweed. Combinations of dicamba + glufosinate provided the most variable results. In the greenhouse, more additive responses were observed in broadleaf weed species compared with the other two mixtures listed previously. Control of monocot species was often synergized by this mixture in the greenhouse. In the field, this tank-mixture often performed antagonistically and significantly reduced the control of annual grass species. Antagonisms were often observed when all three herbicides were applied together in both the greenhouse and the field. However, not all the observed antagonisms resulted in poor control. The results of these studies suggest that growers may see a reduction in control of

important weed species with applications of glufosinate mixed with glyphosate or dicamba. However, under most circumstances, acceptable control of GR waterhemp and horseweed should still be achieved with these tank mixtures.

Nomenclature: Dicamba; glufosinate; glyphosate; soybean, *Glycine max* (L.) Merr. GLXMA; common lambsquarters, *Chenopodium album* L. CHEAL; common ragweed, *Ambrosia artemisiifolia* L. AMBEL; horseweed, *Erigeron canadensis* L. ERICA; Powell amaranth, *Amaranthus powellii* S. Watson AMAPO; waterhemp, *Amaranthus tuberculatus* (Moq.) J.D. Sauer AMATU; Palmer amaranth, *Amaranthus palmeri* S. Watson; barnyardgrass, *Echinochloa crus-galli* (L.) P. Beauv.; yellow foxtail, *Setaria pumila* (Poir.) Roem. & Schult.

Keywords: 3-way resistant soybean; additive; antagonism; dicamba-, glyphosate-, and glufosinate-resistant soybean; glyphosate-resistant horseweed; glyphosate-resistant Palmer amaranth; glyphosate-resistant waterhemp; synergism, weed control systems.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats), waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer), and horseweed (*Erigeron canadensis* L.) have been coined as some of the most troublesome weeds in agronomic row crop production, due to season-long emergence, rapid growth, and prolific seed production (Bhowmik and Bekech 1993; Buhler and Owen 1997; Hager et al. 1997; Horak and Loughin 2000; Keeley et al. 1987; Sellers et al. 2003). In addition to each species' innate biological characteristics, their inherent ability to develop resistance to several herbicide sites of action, including glyphosate, make them difficult to control. In the United States, Palmer amaranth, waterhemp, and horseweed have developed resistance to eight, seven, and five herbicide sites of action, respectively (Heap 2020). The ability of these species to develop resistance to multiple herbicides, including glyphosate, further restricts the number of effective herbicides that can be used for the management of these weeds.

Traditional soybean (*Glycine max* (L.) Merr.) technologies offer farmers limited effective herbicide options for managing tough to control, glyphosate- and multiple-resistant weeds. However, new soybean technologies, such as XtendFlex® soybean (Bayer CropScience, St. Louis, MO), provide farmers with additional postemergence herbicide options for control of multiple-resistant weeds like Palmer amaranth, waterhemp, and horseweed. Farmers are able to apply herbicides containing dicamba (WSSA Group 4), glyphosate (WSSA Group 9), and/or glufosinate (WSSA Group 10) separately or concurrently for weed control in XtendFlex® soybean (Beckie et al. 2019). The ability to apply dicamba and/or glufosinate with this new trait platform provides farmers with two more effective herbicide options to control glyphosate-resistant weeds, both of these herbicides have been proven to control glyphosate-resistant Palmer amaranth, waterhemp, and horseweed (Beyers et al. 2002; Byker et al. 2003; Coetzer et al. 2002; Eubank et al. 2008; Johnson et al. 2010; Kruger et al. 2010; Spaunhorst and Bradley 2013; Steckel et al. 2006).

Combining herbicides is a practice that is often utilized by farmers to increase the spectrum of weed control and to minimize application trips across a field (Hatzios and Penner 1985). Tank-mixing herbicides can also delay the development of herbicide resistance in weed species (Evans et al. 2016; Hugh and Rebound 2009). However, combining multiple herbicides together can also affect the performance of each herbicide. Combinations of two or more herbicides can yield either additive, synergistic, or antagonistic responses (Colby 1967; Flint et al. 1988; Gowing 1960). Such responses are determined by calculating a predicted control value for herbicide mixtures, referred to as the expected value, and comparing it to the actual observed control that the mixture provides. Expected control values for herbicide combinations are calculated by using control values provided by each herbicide applied alone and inputting them into equations developed by Colby (1967), Flint et al. (1988), or Gowing (1960). If the observed value is significantly less than or greater than what is expected, the combination is determined to be antagonistic or synergistic, respectively. If the values of the observed and expected value are equal, the combination is determined to be additive. Previous research has shown that antagonisms can occur when tank-mixing glyphosate and glufosinate (Bethke et al. 2013; Chuah et al. 2008; Meyer et al. 2020a; Meyer et al. 2020b), as well as glyphosate and dicamba (Flint and Barrett 1989; Meyer et al. 2019; O'Sullivan and O'Donovan 1980; Ou et al. 2018); however, such interactions have varied by species, use rates, and weed size. Tank-mixture interactions have not been as clear with mixtures of glufosinate and dicamba, or the three-way mixture of glyphosate, glufosinate, and dicamba.

Although previous research is limited, applications containing combinations of glyphosate and glufosinate are generally accepted to be antagonistic in nature. Bethke et al. (2013) reported early synergisms with applications of glyphosate + glufosinate; however, antagonisms were later documented in the field and greenhouse across several annual broadleaf and grass species. The greatest antagonistic responses occurred on common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.), and giant foxtail (*Setaria faberi* Herrm.) when 240 g ai ha^{-1} of glufosinate was applied with 840 g ae ha^{-1} of glyphosate. Meyer et al. (2020a) reported that glufosinate tank-mixed with glyphosate at multiple rates was antagonistic for barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) control. Likewise, antagonisms in goosegrass (*Eleusine indica* (L.) Gaertn.) control have been documented with varying rates of glyphosate and glufosinate (Chuah et al. 2008). The mechanism by which this antagonism occurs has been attributed to the rapid activity of glufosinate on the plant's photosynthetic systems reducing glyphosate's ability to translocate throughout the plant (Bethke et al. 2013; Chuah et al. 2008; Kudsk and Mathiassen 2004). Researchers have reported reductions in glyphosate absorption and translocation in barnyardgrass, giant foxtail, and Palmer amaranth when glufosinate was tank-mixed with glyphosate (Besancon et al. 2018; Meyer et al. 2020b).

Applications of dicamba tank-mixtures with glyphosate have also been reported to be antagonistic. However, antagonistic responses from this herbicide combination have primarily been reported in monocot species. Although dicamba is considered to have no POST activity on grass species, it appears to influence glyphosate activity and reduce control when added to a mixture. O'Sullivan and O'Donovan (1980) observed a reduction of wild oat (*Avena fatua* L.), barley (*Hordium vulgare* L.), and wheat (*Triticum aestivum* L.) control when dicamba was added to glyphosate. Flint and Barrett (1989) observed similar results with dicamba tank-mixtures with glyphosate on johnsongrass (*Sorghum halepense* (L.) Pers.). Tank-mixtures of dicamba and glyphosate have also reduced barnyardgrass control (Meyer et al. 2015; Meyer et al. 2019; Meyer et al. 2020b). These reductions were more apparent when barnyardgrass was 20 cm versus 8 cm tall (Meyer et al. 2015). Antagonistic responses have also been documented in broadleaf weeds with tank-mixtures of glyphosate and dicamba. Ou et al. (2018) reported reduced translocation of both glyphosate and dicamba in kochia (*Bassia scoparia* (L.) A.J. Scott), resulting in poor control. In contrast, Spaunhorst and Bradley (2013) documented an increase in glyphosate-resistant waterhemp control when glyphosate was added to dicamba.

Limited research has examined interactions of glufosinate and dicamba tank-mixtures. Texas signalgrass (*Urochloa texana* (Buckley) R. Webster) and broadleaf signalgrass (*Urochloa* *platyphylla* (Munro ex C. Wright) R. D. Webster) control was not affected when dicamba was added to glufosinate; however, Palmer amaranth control increased from 76 to 92% when glufosinate was added to dicamba (Merchant et al. 2013). Likewise, Chahal and Johnson (2012) did not observe any differences in glyphosate-resistant horseweed control when dicamba was added to glufosinate. Mixtures of glufosinate and dicamba were deemed to be additive in nature for giant ragweed (*Ambrosia trifida* L.) control in glufosinate-resistant corn (Ganie and Jhala 2017). Although not examined as an interaction, glyphosate-resistant common lambsquarters control increased from 44% with glufosinate alone to 78% when the application contained both glufosinate and dicamba (Chahal and Johnson 2012). In the greenhouse, Joseph et al. (2018) reported a synergistic response in sicklepod (*Senna obtusifolia* (L.) H. S. Irwin & Barneby) control when glufosinate was tank mixed with dicamba. In XtendFlex® cotton (*Gossypium hirsutum* L.), rescue treatments of 590 g ai ha^{-1} glufosinate + 560 g ae ha^{-1} dicamba provided 91% Palmer amaranth control compared with 66% control with glufosinate alone at 590 g ai ha^{-1} (Vann et al. 2017).

Although previous research has identified the potential for herbicide interactions in mixtures of dicamba, glyphosate, and glufosinate, results have varied across species and application rates. Therefore, the objectives of this research were to: 1) characterize the response of various agronomically important weeds to combinations of dicamba, glyphosate, and glufosinate at varying rates in the greenhouse, and 2) evaluate the control of various non-GR weeds, GR waterhemp, and GR horseweed with combinations of dicamba, glyphosate, and glufosinate in the field.

Materials and Methods

Greenhouse Experiments. Greenhouse experiments were conducted at Michigan State University (MSU) in East Lansing, MI in 2019 and 2020 to examine tank-mixture interactions between dicamba, glyphosate, and glufosinate on glyphosate-resistant (GR) and -susceptible (GS) Palmer amaranth, GR and GS waterhemp, GR horseweed, barnyardgrass, and yellow foxtail (*Setaria pumila* (Poir.) Roem. & Schult). Seed sources included confirmed GR Palmer amaranth and GR waterhemp populations collected from Barry (42.702467º N; 85.524992º W) and Isabella County, MI (43.518333º N; 84.664444º W), respectively. Glyphosate-resistant horseweed, barnyardgrass, and yellow foxtail seed were collected from populations at MSU in Ingham County, MI (42.685556º N; 84.488056º W). Glyphosate-susceptible Palmer amaranth and waterhemp populations were sourced from previous experiments at MSU. Waterhemp and Palmer amaranth seed were treated with a 50% sulfuric acid and water solution for 4 min, rinsed, and then exposed to gibberellic acid at a concentration of 0.15 g L^{-1} of water for 6 h to enhance germination.

Plants were grown from seed in 10 x 10 x 12 cm pots filled with potting media (Suremix Perlite, Michigan Grower Products, Inc., Galesburg, MI). Plants were grown in the greenhouse at 25 ± 5º C and sunlight was supplemented to provide total midday light intensity of 1,000 µmol m^{-2} s^{-1} photosynthetic photon flux at plant height in a 16 h day. Plants were watered daily and fertilized as needed to promote optimum plant growth. Palmer amaranth, waterhemp, and horseweed were thinned to one plant per pot and barnyardgrass and yellow foxtail were thinned to two plants per pot.

Dose-Response Experiments. Dose-response experiments were conducted to determine the sensitivity of each weed species to dicamba, glyphosate, and glufosinate. Dicamba and glufosinate were applied at rates ranging from 0.03125 to 2X their standard field use rates. Glyphosate rates examined were based on resistance or sensitivity to glyphosate. Glyphosate-resistant populations were treated with rates ranging from 0.5 to 32X while GS populations were treated with rates ranging from 0.03125 to 2X. Non-treated controls were included for comparison. The 1X rates for dicamba, glyphosate, and glufosinate were 560 g ae ha^{-1}, 1,260 g ae ha^{-1}, and 650 g ai ha^{-1}, respectively. Dicamba, glyphosate, and glufosinate were applied as formulated products; the formulations used were XtendiMax (Bayer CropScience, St. Louis, MO), Roundup PowerMax (Bayer CropScience, St. Louis, MO), and Liberty (BASF Corporation, Research Triangle Park, NC), respectively. Glyphosate and glufosinate treatments were applied with spray grade ammonium sulfate (AMS) at 2% w w^{-1} (Actamaster, Loveland Products, Inc., Loveland, CO). Herbicides were applied to Palmer amaranth, waterhemp, barnyardgrass, and yellow foxtail when weed heights averaged 15 cm tall. Barnyardgrass and yellow foxtail had approximately two to three tillers at the time of herbicide application. Herbicides were applied to horseweed when the average rosette diameter was 8 cm. Herbicide treatments were applied using a single-track sprayer (Generation 4, DeVries Manufacturing, Inc., Hollandale, MN) equipped with an 8001E TeeJet flat-fan nozzle (TeeJet Technologies, Wheaton, IL) calibrated to deliver 187 L ha^{-1} at 193 kPa of pressure. Weed control was evaluated at 7 and 14 DAT using a 0 to 100% scale, where 0 represented no control and 100 equaled complete plant death. Aboveground biomass was harvested at 14 DAT, dried for 7 d at 60° C, and weighed. Dry weights were converted to a percent of the non-treated controls. All treatments were replicated five times and each experiment was repeated in time.

Data were analyzed using non-linear regression in the drc package in R (R version 3.6.0, R Development Core Team 2019). Each weed species was analyzed individually. A three-parameter log-logistic model (Equation 1) was fitted to the data as selected by the drc modelFit function using the lack-of-fit test. The effective dose required to reduce weed biomass by 25 and 50% (ED_{25} and ED_{50}) were determined using the ED function for each weed species.

$$y = c + \frac{d-c}{1+exp\left[b\left(log(x)-log(e)\right)\right]} \qquad \text{[EQ. 1]}$$

For the equation above, *y* is the biomass response (percent of the non-treated control), *x* is the dose, *c* and *d* are the lower and upper limits, respectively, *b* is the relative slope around *e*, and *e* is the ED_{50} (Streibig 1988).

Herbicide Interactions with Dicamba, Glyphosate, and Glufosinate. Dicamba, glyphosate, and glufosinate were applied alone, in paired combinations, and in a three-way mixture at rates equivalent to the ED_{25}, ED_{50}, and 1X rate for each weed species (Table 3.1). The base 1X rates for dicamba, glyphosate, and glufosinate were 560 g ae ha^{-1}, 1,260 g ae ha^{-1}, and 650 g ai ha^{-1}, respectively. If the 1X use rate was used for the ED_{50}, the rate providing 75% reduction in biomass (ED_{75}) was used instead. Paired herbicide combinations were applied in a matrix format so all possible combinations of each herbicide at each rate were applied. Three-way mixtures were applied using combinations of each herbicide at their respective ED_{25}, ED_{50}, and 1X use rates. Glyphosate and glufosinate applied alone and in combination were applied with spray grade AMS at 2% w w^{-1}. There were no adjuvants included with treatments that contained dicamba. Non-treated controls were included for each species as a comparison. Herbicide treatments were applied at similar weed heights using the equipment described previously for the dose-response experiments. Weed control was evaluated at 7 and 14 DAT on a scale of 0 to 100.

Aboveground biomass was harvested, dried, weighed; and dry weights were converted to a percent of the non-treated control. All treatments were repeated five times and each experiment repeated in time.

Data were analyzed using analysis of variance (ANOVA) in the lmer function of R v. 3.6.0 (R Development Core Team 2019). Analysis of variance was conducted to test for the effect of herbicide treatment. Each experiment was analyzed separately. The assumption of normality was checked by examining histogram and normal probability plots of the residuals. Unequal variance assumption was assessed by visual inspection of side-by-side box plots of the residuals followed by the Levene's test for unequal variances. In cases of marked deviations from normality, the data were log-transformed and further analyses were performed using the transformed data. For all experiments, treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$. Biomass data (percent of the non-treated control) was used to determine herbicide interactions. Herbicide combinations were determined to be antagonistic, synergistic, or additive by comparing the observed plant responses with the expected response when the herbicides were combined. Expected biomass reduction values were calculated using Colby's method for paired herbicide combinations or with three-way herbicide mixtures (Equation 2).

$$E = \frac{AB}{100} \quad OR \quad E = \frac{ABC}{10{,}000} \qquad \text{[EQ. 2]}$$

Where *E* is the expected value of the herbicide combination, *A* is the observed biomass reduction by herbicide 1, *B* is the observed biomass reduction by herbicide 2, and *C* is the observed biomass reduction by herbicide 3. It should be noted that values in equation 2 are expressed in percent-of-control, or percent of the non-treated control. The expected and observed values for each herbicide combination were compared using paired t-tests in R v. 3.6.0. If the observed reduction in biomass from a herbicide combination was significantly less than expected the combination was antagonistic; if the observed reduction in biomass was significantly greater than expected the combination was synergistic; and if there was no significant difference between the observed and expected biomass reduction values, the combination was deemed to be additive.

Field Experiments. Three different field experiments were conducted in 2019 and 2020 to evaluate control of GR waterhemp, GR horseweed, and several non-GR weeds in the absence of a crop utilizing POST combinations of dicamba, glyphosate, and glufosinate. The non-GR weed control experiment was conducted in a conventionally tilled field at the MSU Agronomy Farm (MSU-1) in Ingham County, MI (42.685556º N; 84.488056º W). Weeds present at the time of application were annual grasses (including barnyardgrass, giant foxtail (*Setaria faberi* Herrm.), and yellow foxtail); common lambsquarters; common ragweed (*Ambrosia artemisiifolia* L.); and Powell amaranth (*Amaranthus powellii* S. Watson). Weed densities in the non-treated controls were 290/405, 15/12, 8/3, and 12/9 plants m^{-2} for annual grasses, common lambsquarters, common ragweed, and Powell amaranth in 2019/2020, respectively. The GR waterhemp experiment was conducted in a conventionally tilled field in Isabella County (ISB-1), MI (43.518333º N; 84.664444º W). Waterhemp densities at this location were 155 and 162 plants m^{-2} in 2019 and 2020, respectively. The GR horseweed experiment was conducted at the MSU Agronomy Farm (MSU-2) in Ingham County, MI under no-tillage conditions. GR horseweed densities at this location were 235 and 292 plants m^{-2} in 2019 and 2020, respectively. All experiments were arranged in a randomized complete block design with four replications. Plot sizes ranged from 3 m wide x 7.5 to 10 m long.

Dicamba, glyphosate, and glufosinate were applied alone, in paired combinations with each other, and in a three-way mixture. Table 3.2 provides a complete listing of herbicide treatments used in these experiments. Non-treated controls were also included in each experiment. Herbicides were applied when weed heights averaged 10 cm tall using a tractor-mounted compressed air sprayer calibrated to deliver 177 L ha^{-1} at a pressure of 207 kPa through TTI 11003 flat-fan nozzles (TeeJet, Spraying Systems CO., Wheaton, IL 60187).

Weed control was evaluated 7, 14, 21 and 42 days after treatment (DAT). Evaluations were based on a scale of 0 to 100%, with 0 = no control and 100 = complete plant death. Aboveground biomass was harvested 14 and 42 DAT from two random 0.25 m^2 quadrats in each plot. Biomass was dried at 60° C for approximately 1 wk and weighed to calculate biomass reduction as a percent of the non-treated controls.

Statistical Analysis. Statistical analysis was conducted using analysis of variance (ANOVA) in the lmer function of R v. 3.6.0 (R Development Core Team 2019). Analysis of variance was conducted to test for the effect of herbicide treatment along with interactions between site-years (individual year and location). Data were combined over site-year when the interaction of site-year and herbicide treatment was not significant. Each experiment was analyzed separately. The assumption of normality was checked by examining histogram and normal probability plots of the residuals. Unequal variance assumption was assessed by visual inspection of side-by-side box plots of the residuals followed by the Levene's test for unequal variances. In cases of marked deviations from normality, the data were log-transformed and further analyses were performed using the transformed data. For all experiments, treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$.

Biomass data (percent of the non-treated control) were used in determining herbicide interactions. Herbicide combinations were determined to be antagonistic, synergistic, or additive by comparing the observed plant responses with the expected response. Biomass data was subjected to Colby's analysis for paired herbicide combinations or for three-way herbicide mixtures to obtain expected values as percent-of-control (Equation 2) (Colby 1967). Expected visual control evaluations for paired herbicide combinations were calculated using the equation described by Gowing (1960) (Equation 3) while expected visual control evaluations for the three-way herbicide mixture were calculated using the equation described by Colby (1967) (Equation 4).

$$E = A + \left(\frac{B(100-A)}{100}\right) \qquad \text{[EQ. 3]}$$

$$E = A + B + C - \left(\frac{AB+AC+BC}{100}\right) + \frac{ABC}{10{,}000} \qquad \text{[EQ. 4]}$$

Where *E* is the expected value of the herbicide combination, *A* is the observed visual control by herbicide 1, *B* is the observed visual control by herbicide 2, and *C* is the observed visual control by herbicide 3. The expected and observed visual control values for each herbicide combination were compared using paired t-tests in R v. 3.6.0. If the observed visual control value of a herbicide combination was significantly less than or greater than expected, the combination was antagonistic or synergistic, respectively. If the observed visual control was similar to what was expected, the combination was additive.

Results and Discussion

Greenhouse Experiments. *Dose-Response Experiments.* Dose-response experiments were conducted to characterize the response of seven different weed populations to dicamba, glyphosate, and glufosinate. This analysis confirmed the susceptibility and/or resistance of the populations examined. The doses required to reduce biomass for each weed population by 25% and 50% (ED_{25} and ED_{50} values), 14 DAT, can be found in Table 3.1. The glyphosate rates required to reduce GR waterhemp biomass by 50% (ED_{50}) was 630 g ae ha^{-1}, while the ED_{50} for GR Palmer amaranth and GR horseweed were 1,260 g ae ha^{-1}. Glyphosate-susceptible Palmer amaranth and waterhemp showed similar sensitivity to glyphosate with ED_{50} rates of 40 g ae ha^{-1}. The respective R:S ratios of Palmer amaranth and waterhemp to glyphosate were 32 and 16X, respectively (data not shown). The R:S ratio for GR horseweed was unable to be determined as a GS horseweed population could not be sourced. Overall, broadleaf sensitivity to dicamba and glufosinate were similar across all broadleaf weed populations examined in this research. The dicamba rate required to reduce biomass by 50% (35 g ae ha^{-1}) did not differ between GR and GS populations or between broadleaf species examined. Similar trends were observed with glufosinate as the rate required to reduce biomass by 50% (40 g ai ha^{-1}) was the same across all broadleaf populations examined.

Monocot weed species are generally tolerant of POST applications of dicamba (Flint and Barret 1989; O'Sullivan and O'Donovan 1980). For this reason, herbicide rate selection was based around the 1X application rate of 560 g ae ha^{-1}. Dicamba rates used on monocot species were 280 g ae ha^{-1}, 560 g ae ha^{-1}, and 1,120 g ae ha^{-1}, or 1/2X, 1X, and 2X field use rates. Greater biomass reductions were observed in barnyardgrass with applications of dicamba compared with yellow foxtail. Dicamba rates of 560 and 1,120 g ae ha^{-1} resulted in reductions of barnyardgrass biomass by 16% and 36%, respectively, while yellow foxtail biomass was only reduced by 8% and 19%, respectively. Sensitivity to glyphosate was similar between barnyardgrass and yellow foxtail as the rate required to reduce biomass by 50% was 80 g ae ha^{-1} for both species. Yellow foxtail was slightly more sensitive to glufosinate compared with barnyardgrass. The glufosinate rate to reduce yellow foxtail biomass by 50% was 80 g ai ha^{-1} compared with 160 g ai ha^{-1} for barnyardgrass.

Herbicide Interactions with Dicamba, Glyphosate, and Glufosinate. All tank-mixtures of glyphosate + glufosinate or glyphosate + dicamba were antagonistic in their control of GS Palmer amaranth; the observed level of growth reduction for these combinations were significantly less than what was expected using Colby's method (Figures 3.1A and 3.1B). Glyphosate-susceptible Palmer amaranth biomass was reduced 93% by glyphosate at 40 g ae ha^{-1} and 85% by glufosinate at 40 g ai ha^{-1}; the combination of these herbicide rates only reduced biomass by 72% (Figure 3.1A). The addition of 35 g ae ha^{-1} dicamba to glyphosate only reduced GS Palmer amaranth biomass by 79% (Figure 3.1B). Similar trends were observed when 20 g ae ha^{-1} glyphosate was applied with 9 g ae ha^{-1} dicamba. Seven out of the nine dicamba + glufosinate tank-mixtures were antagonistic for GS Palmer amaranth control (Figure 3.1C). The two remaining treatments dicamba at 35 g ae ha^{-1} + glufosinate at either 20 or 40 g ai ha^{-1} were deemed to be additive. Although antagonistic responses were observed, reductions in GS Palmer amaranth growth were 92% or higher when the highest rates of glyphosate (1260 g ae ha^{-1}), glufosinate (650 g ai ha^{-1}), or dicamba (560 g ae ha^{-1}) were applied alone or in combination of lower rates of any of these herbicides.

Similar to GS Palmer amaranth, all combinations of glyphosate + glufosinate were antagonistic for GR Palmer amaranth control (Figure 3.1D). The GR Palmer amaranth population experienced significantly lower reductions in biomass when glyphosate at 2,520 g ae ha^{-1} was combined with glufosinate at either 20 or 40 g ai ha^{-1}, providing 77-78% control compared with glyphosate applied alone at 2,520 g ae ha^{-1}, which provided 90% control. However, when glufosinate was applied at the 1X rate of 650 g ai ha^{-1}, maximum reductions in biomass were observed even though antagonisms were observed. Most combinations of dicamba

+ glyphosate were antagonistic (Figure 3.1E). However, the combinations of glyphosate at 630 g ae ha^{-1} + dicamba at either 9 or 35 g ae ha^{-1} performed as expected. The combination of glyphosate at 2,520 g ae ha^{-1} + dicamba at 9 g ae ha^{-1} was also additive. Combinations of dicamba + glyphosate that contained the highest rate of either dicamba or glyphosate provided the greatest reductions in biomass. Although many of these treatments were deemed antagonistic, their control of GR Palmer amaranth did not differ from the top performing treatments. Combinations of dicamba and glufosinate were generally additive in controlling GR Palmer amaranth (Figure 3.1F). Only three treatments resulted in observed antagonisms. Two of these antagonisms were observed when glufosinate at 650 g ai ha^{-1} was mixed with dicamba at either 9 or 560 g ae ha^{-1}. However, these two treatments provided similar control of GR Palmer amaranth compared with glufosinate at 60 g ai ha^{-1} applied alone and were not different than the top performing treatments. The remaining antagonism occurred with glufosinate at 40 g ai ha^{-1} + dicamba at 9 g ae ha^{-1} which reduced biomass by 47% compared with 63 and 38% from glufosinate and dicamba applied alone, respectively.

In general, combinations of glyphosate + glufosinate, dicamba + glyphosate, and dicamba + glufosinate were antagonistic in control of GS waterhemp (Figures 3.2A, 3.2B, and 3.2C). The only combination of glyphosate + glufosinate that was additive was glyphosate at 20 g ae ha^{-1} + glufosinate at 40 g ai ha^{-1}, which provided 81% control of GS waterhemp (Figure 3.2A). However, when the glyphosate rate in this mixture was increased from 20 to 40 g ae ha^{-1} GS waterhemp biomass was reduced to 52%. Similarly, the combination of glyphosate at 20 g ae ha^{-1} + dicamba at 560 g ae ha^{-1} also reduced GS waterhemp biomass as expected (Figure 3.2B). The combination of glyphosate at 40 g ae ha^{-1} + dicamba at 9 g ae ha^{-1} significantly reduced control compared with the application of glyphosate at 40 g ae ha^{-1} applied alone. Similarly, the addition of glyphosate at 20 g ae ha^{-1} to dicamba at 9 g ae ha^{-1} reduced GS waterhemp growth to 31% compared with dicamba alone at 53%. Combinations of dicamba and glufosinate that were additive included glufosinate at 20 g ai ha^{-1} + dicamba with either 35 or 560 g ae ha^{-1} (Figure 3.2C). Similar to GS Palmer amaranth, GS waterhemp maximum biomass reduction was achieved when at least one herbicide in any tank-mixture was applied at a 1X rate. Although antagonistic responses were observed, reductions in GS waterhemp growth were 94% or higher when the highest rates of glyphosate (1260 g ae ha^{-1}), glufosinate (650 g ai ha^{-1}), or dicamba (560 g ae ha^{-1}) were applied alone or in combination of lower rates of any of these herbicides.

Similar to the GS waterhemp population, combinations of glyphosate + glufosinate, dicamba + glyphosate, and dicamba + glufosinate were generally antagonistic in controlling GR waterhemp (Figures 3.2D, 3.2E, and 3.2F). Glyphosate at 158 g ae ha^{-1} reduced GR waterhemp biomass by 47% and glufosinate at 20 g ai ha^{-1} reduced biomass by 36%; however, the combination of these herbicide rates only reduced biomass by 24% (Figure 3.2D). The only combination that was additive was glyphosate at 630 g ae ha^{-1} + glufosinate at 40 g ai ha^{-1}. Similarly, many of the glyphosate + dicamba showed an antagonistic response. The combinations of glyphosate at 630 g ae ha^{-1} + dicamba at either 9 or 35 g ae ha^{-1} were the only glyphosate + dicamba mixtures that performed as expected (Figure 3.2E). In one instance, GR waterhemp control was greater with a combination of glyphosate at 158 g ae ha^{-1} + dicamba at 35 g ae ha^{-1} compared with either herbicide applied alone. However, this combination was still determined to be antagonistic. Combinations of dicamba at all examined rates + glufosinate at 20 g ai ha^{-1} reduced GR waterhemp biomass as expected (Figure 3.2F). The remaining dicamba + glufosinate treatments were antagonistic. Greater than 90% reductions in biomass were achieved with herbicide combinations that contained at least one herbicide at its respective 1X rate with the exception of glyphosate at 1,260 g ae ha^{-1} applied alone (89%) and with glufosinate at 20 g ai ha^{-1} (84%). Similar to GS waterhemp, maximum GR waterhemp biomass reduction was achieved when at least one herbicide in any tank-mixture was applied at a 1X rate. Although antagonistic responses were observed, reductions in GR waterhemp growth were 84% or higher when the highest rates of glyphosate (1260 g ae ha^{-1}), glufosinate (650 g ai ha^{-1}), or dicamba (560 g ae ha^{-1}) were applied alone or in combination of lower rates of any of these herbicides.

Overall reductions in horseweed biomass from examined treatments were slightly lower than Palmer amaranth or waterhemp; only one treatment reduced biomass 90%. With the exception of one glyphosate + glufosinate treatment, all combinations of glyphosate + glufosinate, dicamba + glyphosate, and dicamba + glufosinate were antagonistic in controlling GR horseweed (Figures 3.3A, 3.3B, and 3.3C). When glyphosate at 1,260 g ae ha^{-1} was combined with glufosinate at 20 g ai ha^{-1}, control of GR horseweed was greater than either herbicide applied alone at such rates (Figure 3.3A). However, this combination was determined to be antagonistic as the observed control value was significantly less than expected. The only additive combination was glyphosate at 158 g ae ha^{-1} + glufosinate at 40 g ai ha^{-1}. This treatment was also the only treatment to reduce GR horseweed biomass by 90%.

Few antagonisms were observed for barnyardgrass control in the greenhouse with tank mixtures of glyphosate + glufosinate, dicamba + glyphosate, and dicamba + glufosinate (Figures 3.4A, 3.4B, and 3.4C). Applications of glyphosate + glufosinate were generally additive in controlling barnyardgrass (Figure 3.4A). Glyphosate at 1,260 g ae ha^{-1} applied alone reduced barnyardgrass biomass by 86% while applications of glufosinate at 160 g ai ha^{-1} reduced biomass by 50%; however, the combination of these herbicide rates only reduced biomass by 86%. Due to the lack of increased control from this tank mixture, this combination was determined to be antagonistic. The only other observed antagonism on barnyardgrass control was with the combination of glyphosate at 1,260 g ae ha^{-1} + glufosinate at 650 g ai ha^{-1}, which provided a 92% reduction in biomass. A synergism was observed with an application containing glyphosate at 80 g ae ha^{-1} + glufosinate at 80 g ai ha^{-1}. When these herbicide rates were applied alone, glyphosate and dicamba reduced barnyardgrass biomass 17 and 15%, respectively. However, the combination of these two herbicides rates reduced biomass by 65%, a response that was significantly greater than expected. Bethke et al. (2013) observed synergisms in the control of giant foxtail with certain combinations of glyphosate + glufosinate at reduced rates. In contrast, Meyer et al (2020a) observed antagonisms in the control of barnyargrass with various mixtures of glyphosate and glufosinate. The addition of dicamba to glyphosate resulted in additive responses will all treatments examined (Figure 3.4B). When dicamba was combined with glyphosate at 1,260 g ae ha^{-1}, control ranged from 86 to 90%. However, when the glyphosate rate was lowered to 40 or 80 g ae ha^{-1}, control was reduced to 48% or less. No antagonisms were observed with combinations of dicamba + glufosinate (Figure 3.4C). Five of the nine treatments examined were additive while the remaining four treatments were synergistic in their control of barnyardgrass. Synergisms in control were observed when dicamba at all examined rates was combined with glufosinate at 80 g ai ha^{-1}. The remaining synergism occurred with dicamba at 560 g ae ha^{-1} + glufosinate at 160 g ai ha^{-1}. Barnyardgrass control was maximized with tank-mixtures that contained either glyphosate or glufosinate at their respective 1X rates.

As with barnyardgrass, combinations of glyphosate + glufosinate, dicamba + glyphosate, and dicamba + glufosinate were generally additive for yellow foxtail control (Figures 3.5A, 3.5B, and 3.5C). Antagonisms were observed with combinations of glyphosate at all rates examined + glufosinate at 650 g ai ha^{-1} as well as the mixture of glyphosate at 1,260 g ae ha^{-1} +

glufosinate at 80 g ai ha^{-1} (Figure 3.5A). Although these antagonisms were observed, control of yellow foxtail with these treatments were similar to the control provided by glyphosate at 1,260 g ae ha^{-1} or glufosinate at 650 g ai ha^{-1} applied alone. Only two combinations of dicamba + glyphosate were determined to be antagonistic; the remaining treatments were additive in nature (Figure 3.5B). The combination of glyphosate at 1,260 g ae ha^{-1} + dicamba at either 560 or 1,120 g ae ha^{-1} were antagonistic. No antagonisms were observed with the combination of dicamba + glufosinate for yellow foxtail control (Figure 3.5C). Synergisms were observed with three treatments: glufosinate at 40 g ai ha^{-1} + dicamba at 1,120 g ae ha^{-1} and glufosinate at 80 g ai ha^{-1} + dicamba at either 560 or 1,120 g ae ha^{-1}. Similar to barnyardgrass, yellow foxtail biomass reductions were greatest with treatments that utilized either glyphosate or glufosinate at their respective 1X rates.

Three-way mixtures of dicamba, glyphosate, and glufosinate were also examined on the weed populations previously mentioned (Table 3.3). In general, the combination of these three herbicides together resulted in antagonistic responses, especially on the broadleaf weeds examined. The only additive response in a broadleaf weed species was when GR Palmer amaranth was treated with dicamba, glyphosate, and glufosinate combined at rates equivalent to each herbicide’s ED_{25} value. Additive responses were also observed with barnyardgrass and yellow foxtail. The combination of these three herbicides at their respective ED_{25} rates performed as expected in yellow foxtail while treatments combining each herbicide at their respective ED_{50} rates were additive in both barnyardgrass and yellow foxtail. There was one observed synergism with the three-way mixture of dicamba, glyphosate, and glufosinate. This synergism was observed with barnyardgrass treated with the three herbicides mixed at their appropriate ED_{25} rates; the expected control was only 15% while the actual reduction in biomass was 66%.

Overall, antagonisms with various combinations of dicamba, glyphosate, and glufosinate were frequently observed in broadleaf weed species. Combinations of glyphosate + glufosinate and dicamba + glyphosate appeared to have the greatest frequency of antagonistic responses in these broadleaf weeds. Glyphosate-resistant horseweed appeared to be most prone to antagonistic responses with any of the examined tank-mixtures. In contrast, few antagonisms were observed in the monocot species examined. Synergisms in barnyardgrass and yellow foxtail were most common with combinations of dicamba + glufosinate. It should be noted that although certain treatments were antagonistic, they may not have necessarily provided control that was different than the top performing treatments. In many instances, especially with combinations utilizing the highest rates for each herbicide, the expected biomass reductions from Colby's method were greater than the maximum observed biomass reduction from any treatment. Such instances were often determined to be antagonistic.

Field Experiments. Control of common lambsquarters, common ragweed, and Powell amaranth at MSU-1 were greater than 96% with all tank-mixtures examined, 14 DAT (Table 3.4). Applications of dicamba alone only provided 58 to 87% control while applications of glyphosate alone and glufosinate alone provided greater than 99% and greater than 89% control of these weeds, respectively. Control of common lambsquarters improved with the combination of dicamba + glyphosate compared with either herbicide applied alone. Merchant et al. (2013) also observed an increase in the control of common lambsquarters with applications of dicamba + glufosinate compared with glufosinate alone. All paired herbicide combinations were additive, 14 DAT, with the exception of dicamba + glyphosate + glufosinate, which was antagonistic for Powell amaranth control. However, control of Powell amaranth with this tank-mixture was still acceptable (96%).

By 42 DAT, control of common lambsquarters, common ragweed, and Powell amaranth were greater than 90% with three out of the four tank-mixtures examined (Table 3.5). The combination of glyphosate + glufosinate was the only mixture to provide less than 90% control of these species, 42 DAT. Glyphosate + glufosinate were antagonistic for common lambsquarters and Powell amaranth control. Although common ragweed control was lower than what was expected, the difference in control was not significant and so the mixture was additive ($p = 0.05388$). Antagonisms between glyphosate and glufosinate in controlling broadleaf weeds have been documented in previous literature (Besancon et al. 2018; Bethke et al. 2013). Antagonisms were also observed with dicamba + glyphosate on common lambsquarters and Powell amaranth control. However, overall control of these weeds was greater than 94% and were not different than other treatments that provided the greatest control of these species. Antagonisms were also documented in Powell amaranth control with applications of dicamba + glufosinate, as well as with the three-way herbicide mixture. Similar to applications of dicamba + glyphosate, these tank-mixtures also provided excellent control of Powell amaranth and were not different than the top performing treatments.

Site-year had a significant effect ($p \leq 0.05$) on herbicide treatment for annual grass control; therefore, data were analyzed separately by site-year. In 2019, annual grass control was greater than 94% with all treatments, except for dicamba applied alone (0%) and the combination of dicamba + glufosinate, 14 DAT (Table 3.6). The addition of dicamba to applications of glufosinate reduced annual grass control to 88% compared with glufosinate applied alone, which provided 94% control. This combination was determined to be antagonistic. All other tank-mixtures examined were additive, 14 DAT. However, by 42 DAT, antagonisms were observed with three out of the four examined tank-mixture combinations. The combination of dicamba + glyphosate was the only treatment to perform as expected. Applications of dicamba + glufosinate provided 45% control of annual grasses, 42 DAT. The addition of glyphosate to this mixture improved control to 83%. However, when glufosinate was dropped from this mixture and dicamba + glyphosate was applied annual grass control increased further to 91%. Annual grass species, including barnyardgrass, giant foxtail, and yellow foxtail, were the dominant weed at MSU-1. For this reason, plot biomass was greatly influenced by control of these annual grass species. Similar trends were observed with plot biomass 42 DAT as were visual control evaluations at the same time. Antagonisms on biomass reduction were observed with all tank-mixture combinations, except for dicamba + glyphosate. However, applications of glyphosate + glufosinate along with applications of the three-way herbicide mixture, provided similar reductions in biomass as the top performing treatments.

In 2020, applications of glufosinate resulted in lower control of annual grass species, both 14 and 42 DAT (39 and 13%, respectively) (Table 3.7). The addition of dicamba to glufosinate reduced annual grass control 14 DAT from 39 to 21%. Even though the addition of glyphosate to this mixture improved control to 84%, antagonisms in grass control were still observed. Annual grass control 14 DAT was greater than 90% with applications of glyphosate, glyphosate + glufosinate, and dicamba + glyphosate. Although overall control of annual grass was lower, similar trends existed at 42 DAT. Antagonisms were observed with applications of dicamba + glufosinate and with the three-way mixture. The addition of glyphosate to an application of dicamba + glufosinate increased annual grass control from 4 to 19%. Glyphosate applied alone provided the greatest control (49%). Poor late-season control of annual grass species in 2020 is attributed to rapid regrowth and new germination shortly after application. Antagonisms in biomass reductions were observed with applications of glyphosate + glufosinate as well as the three-way mixture. Although the combination of dicamba + glufosinate was not determined to be antagonistic on biomass reduction, the addition of dicamba to an application of glufosinate significantly reduced overall reductions in plot biomass from 29 to 0%.

Applications of glyphosate + glufosinate have been reported to be antagonistic on the control of annual grass species. Chuah et al. (2008) observed antagonisms in goosegrass control when tank-mixing glyphosate and glufosinate together at various rates in the greenhouse. Likewise, Bethke et al. (2013) observed antagonisms in control of giant foxtail in the field and in the greenhouse with multiple combinations of glyphosate + glufosinate. Although antagonisms on control of monocot species have been reported with previous literature (Flint and Barrett 1989; Meyer et al. 2015; Meyer et al. 2019; Meyer et al. 2020b; O'Sullivan and O'Donovan 1980), we observed no antagonistic effects with this combination on annual grass control. Reductions in control of annual grass species with combinations of dicamba + glufosinate were evident. However, limited research on the interaction between these herbicides exist.

Glyphosate applied alone was the least effective treatment for horseweed control at MSU-2 (Table 3.8). Control of horseweed with glyphosate applied alone was less than 15%, 14 and 42 DAT. Prior to the development of glyphosate resistance, POST applications of glyphosate have typically provided excellent control of horseweed (Buhler and Owen 1997; Scott et al. 1998). Glufosinate applied alone provided the greatest control of horseweed 14 DAT (92%), but by 42 DAT control was unacceptable (39%). In contrast, applications of dicamba initially provided poor control of horseweed (62%), but by 42 DAT control was 90%. The addition of glyphosate to glufosinate reduced control of horseweed, 14 and 42 DAT, compared with glufosinate applied alone. This combination was determined to be antagonistic at 42 DAT. Similarly, the addition of dicamba to glufosinate reduced horseweed control compared with glufosinate applied alone. However, by 42 DAT horseweed control with this mixture was significantly greater than the control observed with glufosinate applied alone. Although horseweed control was greater with an application of dicamba + glufosinate than with an application of glufosinate, control was reduced when compared with an application of dicamba applied alone, 42 DAT. Antagonisms were observed with the combination of dicamba + glufosinate at both 14 and 42 DAT. In contrast, Chahal and Johnson (2012) observed similar control of glyphosate-resistant horseweed when dicamba was added to glufosinate. The addition of glyphosate to this mixture did not change the overall control of horseweed at 14 or 42 DAT compared with an application of dicamba + glufosinate. This three-way mixture of dicamba + glyphosate + glufosinate was also determined to be antagonistic at 14 and 42 DAT. Applications of dicamba + glyphosate provided the lowest horseweed control compared with the other tank-mixtures examined, 14 DAT. However, by 42 DAT, this tank-mixture provided the greatest control of horseweed (96%) and was synergistic. Treatments that contained dicamba provided 82 to 96% control of horseweed 42 DAT. Similarly, reductions in horseweed biomass at the same point in time were greatest with these same dicamba-containing treatments (77 to 86%), although some antagonisms were observed. The improved late-season horseweed control from applications containing dicamba compared with glufosinate is likely a result of the soil residual control of newly emerged horseweed that is provided by dicamba.

Similar to horseweed, glyphosate applied alone provided the lowest control of waterhemp at ISB-1 both 14 and 42 DAT (Table 3.9). Dicamba applied alone provided 79% control 14 DAT while the remaining treatments provided 88 to 91% control. Antagonisms in waterhemp control were observed with all tank-mixtures, 14 DAT, with the exception of dicamba + glyphosate. Applications of glufosinate with or without glyphosate provided the greatest reductions in waterhemp biomass, 14 DAT. Applications of glyphosate applied alone, dicamba applied alone, and the combination dicamba + glyphosate reduced waterhemp biomass by 56, 71, and 77%, respectively. The remaining two tank-mixtures, dicamba + glufosinate and dicamba + glyphosate + glufosinate, provided similar reductions in waterhemp biomass, 84 and 88%, respectively. However, these reductions in waterhemp biomass were significantly less than what was expected and therefore were antagonistic.

Similar to horseweed, treatments containing dicamba provided greater than 85% control of waterhemp 42 DAT (Table 3.9). The remaining treatments provided poor control. Antagonisms in waterhemp control were observed with three out of the four tank-mixtures examined, 42 DAT. The remaining tank-mixture, dicamba + glyphosate, provided greater control of waterhemp than was expected, demonstrating a synergistic response. This synergism was also observed in the overall reduction of waterhemp biomass at the same point in time. These results support research conducted by Spaunhorst and Bradley (2013) which observed an increase in control of GR waterhemp with applications of dicamba + glyphosate compared with dicamba applied alone. With the exception of glyphosate applied alone (41%), all of the treatments examined reduced overall waterhemp biomass by 83% or more, 42 DAT. Tank-mixtures that contained dicamba reduced waterhemp biomass by 93% to 99%.

The improved control of both horseweed and waterhemp with the addition of glyphosate to dicamba was unexpected, since these weeds are resistant to glyphosate. These results were also unexpected since the combination of dicamba + glyphosate was often deemed antagonistic in the greenhouse. Antagonisms were also frequently observed with applications of dicamba + glufosinate in both monocot and dicot species. These field results contrast both the greenhouse research conducted and previous research; which has documented this tank-mixture to provide similar or greater control of such weed species (Chahal and Johnson 2012; Ganie and Jhala 2017; Merchant et al. 2013). The addition of glyphosate to the tank-mixture of dicamba + glufosinate often resulted in similar control and was typically unable to overcome the observed antagonisms. The combination of glyphosate + glufosinate were typically additive in these field studies; however, antagonisms were also observed, especially later in the growing season, supporting previous research (Bethke et al. 2013; Meyer et al. 2020a). Overall differences in results between greenhouse and field experiments, especially with combinations containing glufosinate, are likely a result of the growing conditions present within the greenhouse. Consistently high growing temperatures in the greenhouse coupled with high humidity and intense supplemental light are likely responsible for the increased efficacy of glufosinate in the greenhouse.

From this research, we were able to identify various herbicide interactions with combinations of dicamba, glyphosate, and glufosinate that could be used in XtendFlex® soybean. Applications of glufosinate or dicamba provide various benefits to the grower. Applications containing glufosinate often provide rapid control of emerged weeds, but control into the growing season is often limited. Applications containing dicamba typically took longer to provide acceptable control but also provided control of broadleaf weed species later into the growing season. Farmers should consider the weeds present in their fields prior to making a herbicide application in XtendFlex® soybean. Applications of glyphosate + glufosinate are often antagonistic in their control of weedy species and higher rates should be used to help overcome such responses. Applications of dicamba + glyphosate have been reported to be antagonistic in monocot species, but from this research, control of grassy weeds was found to be additive. This combination also resulted in the greatest control of GR horseweed and waterhemp in the field. Applications of dicamba + glufosinate should be avoided in fields with high populations of annual grass species as we observed severe reductions in grass control with this tank-mixture. In many cases, tank-mixture antagonisms did not always result in significantly reduced weed control. Farmers should be aware of these potential herbicide interactions and avoid conditions that may exaggerate them such as cool temperatures and large weeds (Anderson et al. 1993; Burke et al. 2002; Green 1989; Takano and Dayan 2020).

APPENDIX

APPENDIX

CHAPTER III TABLES

Table 3.1. Dicamba, glyphosate, and glufosinate rates for seven different weed populations in the greenhouse based on aboveground biomass reduction. Rates were determined via dose-response experiments. ED_{25} and ED_{50} rates were rounded to the closest rate examined in the dose-response experiments.

	Dicamba[b]			Glyphosate			Glufosinate		
Weed population[a]	ED_{25}	ED_{50}	1X	ED_{25}	ED_{50}	1X[c]	ED_{25}	ED_{50}	1X
	g ae ha^{-1}			g ae ha^{-1}			g ai ha^{-1}		
GS Palmer amaranth	9	35	560	20	40	1,260	20	40	650
GR Palmer amaranth	9	35	560	630	1,260	2,520	20	40	650
GS waterhemp	9	35	560	20	40	1,260	20	40	650
GR waterhemp	9	35	560	158	630	1,260	20	40	650
GR horseweed	9	35	560	158	1,260	15,120	20	40	650
Barnyardgrass	280	560	1,120	40	80	1,260	80	160	650
Yellow foxtail	280	560	1,120	40	80	1,260	40	80	650

[a] GS = glyphosate-susceptible; GR = glyphosate-resistant.

[b] See Table 3.2 for herbicide product information.

[c] For glyphosate-resistant populations where the 1X glyphosate rate (1,260 g ae ha^{-1}) was utilized as the ED_{50}, the 1X glyphosate rate was substituted with a rate of glyphosate that provided 75% control (ED_{75}).

Table 3.2. Herbicide treatments for non-crop field experiments examining herbicide interactions at two locations in Ingham County, MI and one location in Isabella County, MI (2019-2020).

Herbicide treatment	Rates	Trade name	Manufacturer[a]	Adjuvants[b]
	– kg ai or ae ha^{-1} –			
glyphosate	1.26	Roundup PowerMax	Bayer CropScience	AMS
glufosinate	0.65	Liberty	BASF Corporation	AMS
dicamba	0.56	XtendiMax	Bayer CropScience	CAR + DRA
glyphosate + glufosinate	1.26 + 0.65	Roundup PowerMax + Liberty	Bayer + BASF	AMS
dicamba + glyphosate	0.56 + 1.26	XtendiMax + Roundup PowerMax	Bayer + Bayer	CAR + DRA
dicamba + glufosinate	0.56 + 0.65	XtendiMax + Liberty	Bayer + BASF	MON
dicamba + glyphosate + glufosinate	0.56 + 1.26 + 0.65	XtendiMax + Roundup PowerMax + Liberty	Bayer + Bayer + BASF	MON

[a] Manufacturer information: Bayer CropScience, St. Louis, MO, www.cropscience.bayer.com; BASF Corporation, Research Triangle Park, NC, www.basf.com.

[b] Adjuvant information: AMS = ammonium sulfate at 2% w w^{-1} (Actamaster, Loveland Products Inc., Loveland, CO); CAR = non-ammonium sulfate water conditioner at 1% v v^{-1} (Class Act Ridion, WinField United, Arden Hills, MN); DRA = drift reduction agent at 0.5% v v^{-1} (Intact, Precision Laboratories, Waukegan, IL); MON = MON 301471 at 1.5% v v^{-1} (Bayer CropScience, St. Louis, MO).

Table 3.3. Growth reduction of seven different weed populations in the greenhouse with applications of dicamba, glyphosate, and glufosinate in various 3-way mixtures, 14 DAT.

	Herbicide rate (g ae or ai ha^{-1})			Growth reduction (%)			
Population[a]	Dicamba	Glyphosate	Glufosinate	Expected[b]	Observed	P value[c]	Response
GS Palmer amaranth	9	20	20	99	79	<0.0001	Antagonistic
	35	40	40	99	91	0.0041	Antagonistic
	560	1,260	650	99	94	<0.0001	Antagonistic
GR Palmer amaranth	9	630	20	86	78	0.2567	Additive
	35	1,260	40	98	90	0.0006	Antagonistic
	560	2,520	650	99	92	<0.0001	Antagonistic
GS waterhemp	9	20	20	83	55	<0.0001	Antagonistic
	35	40	40	97	78	0.0002	Antagonistic
	560	1,260	650	99	92	<0.0001	Antagonistic
GR waterhemp	9	158	20	89	70	0.0008	Antagonistic
	35	630	40	97	90	0.0013	Antagonistic
	560	1,260	650	99	92	<0.0001	Antagonistic
GR horseweed	9	158	20	89	67	0.0042	Antagonistic
	35	1,260	40	96	78	0.0007	Antagonistic
	560	15,120	650	99	89	0.0003	Antagonistic
Barnyardgrass	280	40	80	15	66	0.0491	Synergistic
	560	80	160	66	82	0.1372	Additive
	560	1,260	650	99	90	<0.0001	Antagonistic
	1,120	1,260	650	99	92	<0.0001	Antagonistic
Yellow foxtail	280	40	40	22	51	0.0966	Additive
	560	80	80	47	64	0.1260	Additive
	560	1,260	650	98	89	0.0002	Antagonistic
	1,120	1,260	650	98	89	<0.0001	Antagonistic

[a] GS = glyphosate-susceptible; GR = glyphosate-resistant

[b] Expected values were calculated using the equation $E = \frac{ABC}{10{,}000}$ as explained by Colby (1967).

[c] P values ≤ 0.05 are indicative of a significant response.

Table 3.4. Activity of dicamba, glyphosate, and glufosinate applied alone, in paired combinations, and in a three-way mix on common lambsquarters, common ragweed, and Powell amaranth in the field (MSU-1) at 14 DAT. Date were combined over years.

	Weed control (14 DAT)								
	Common lambsquarters			Common ragweed			Powell amaranth		
Treatment[a]	Expected[b]	Observed[c]		Expected	Observed		Expected	Observed	
	%								
glyphosate + AMS		100	a[d]		99	a		100	a
glufosinate + AMS		93	b		89	a		96	b
dicamba + CAR + DRA		87	c		77	b		58	c
glyphosate + glufosinate + AMS	100	97	a	100	99	a	100	99	ab
dicamba + glyphosate + CAR + DRA	100	100	a	99	99	a	100	99	ab
dicamba + glufosinate + MON	99	97	a	97	100	a	98	100	a
dicamba + glyphosate + glufosinate + MON	100	99	a	100	98	a	100	96	b (-)

[a] See table 3.2 for herbicide and adjuvant product information and rates.

[b] Expected control values were calculated using Gowing (1960) equation $E = A + (\frac{B(100-A)}{100})$ for paired combinations and Colby (1967) equation $E = A + B + C - \left(\frac{AB+AC+BC}{100}\right) + \left(\frac{ABC}{10{,}000}\right)$ for three-way mixtures.

[c] Antagonisms or synergisms are denoted by (-) or (+), respectively; if no notation is present, mixtures were additive at $\alpha \leq 0.05$.

[d] Means followed by the same letter are not statistically different at $\alpha \leq 0.05$.

Table 3.5. Activity of dicamba, glyphosate, and glufosinate applied alone, in paired combinations, and in a three-way mix on common lambsquarters, common ragweed, and Powell amaranth in the field (MSU-1) at 42 DAT. Date were combined over years.

	Weed control (42 DAT)					
	Common lambsquarters		Common ragweed		Powell amaranth	
Treatment[a]	Expected[b]	Observed[c]	Expected	Observed	Expected	Observed
	%					
glyphosate + AMS		85 bc[d]		92 c		85 b
glufosinate + AMS		80 c		96 a-c		87 b
dicamba + CAR + DRA		95 a		98 ab		93 a
glyphosate + glufosinate + AMS	96	86 b (-)	99	83 d	98	84 b (-)
dicamba + glyphosate + CAR + DRA	99	97 a (-)	100	100 a	99	94 a (-)
dicamba + glufosinate + MON	99	96 a	100	99 ab	99	93 a (-)
dicamba + glyphosate + glufosinate + MON	99	96 a	100	94 bc	99	95 a (-)

[a] See table 3.2 for herbicide and adjuvant product information and rates.

[b] Expected control values were calculated using Gowing (1960) equation $E = A + (\frac{B(100-A)}{100})$ for paired combinations and Colby (1967) equation $E = A + B + C - \left(\frac{AB+AC+BC}{100}\right) + \left(\frac{ABC}{10,000}\right)$ for three-way mixtures.

[c] Antagonisms or synergisms are denoted by (-) or (+), respectively; if no notation is present, mixtures were additive at α ≤ 0.05.

[d] Means followed by the same letter are not statistically different at α ≤ 0.05.

Table 3.6. Activity of dicamba, glyphosate, and glufosinate applied alone, in paired combinations, and in a three-way mix on annual grass species in the field (MSU-1) in 2019. Experiment biomass consisted mainly of annual grass species.

	Annual grass[a] control								Biomass[e]		
	14 DAT				42 DAT				42 DAT		
Treatment[b]	Expected[c]	Observed[d]			Expected	Observed			Expected[f]	Observed	
	%								% reduction		
glyphosate + AMS		100	a[g]			93	a			97	a
glufosinate + AMS		94	c			59	c			68	b
dicamba + CAR + DRA		0	e			0	e			0	d
glyphosate + glufosinate + AMS	100	97	b		97	83	b (-)		99	86	a (-)
dicamba + glyphosate + CAR + DRA	100	99	ab		93	91	a		96	96	a
dicamba + glufosinate + MON	94	88	d (-)		59	45	d (-)		65	39	c (-)
dicamba + glyphosate + glufosinate + MON	100	98	b		97	83	b (-)		99	89	a (-)

[a] Annual grass species consisted of barnyardgrass, giant foxtail, and yellow foxtail.

[b] See table 3.2 for herbicide and adjuvant product information and rates.

[c] Expected control values were calculated using Gowing (1960) equation $E = A + (\frac{B(100-A)}{100})$ for paired combinations and Colby (1967) equation $E = A + B + C - \left(\frac{AB+AC+BC}{100}\right) + \left(\frac{ABC}{10{,}000}\right)$ for three-way mixtures.

[d] Antagonisms or synergisms are denoted by (-) or (+), respectively; if no notation is present, mixtures were additive at $\alpha \leq 0.05$.

[e] Experiment biomass consisted mainly of annual grass species.

[f] Expected biomass reductions were calculated using Colby (1967) equation $E = \frac{AB}{100}\ \ OR\ \ E = \frac{ABC}{10{,}000}$

[g] Means followed by the same letter are not statistically different at $\alpha \leq 0.05$.

Table 3.7. Activity of dicamba, glyphosate, and glufosinate applied alone, in paired combinations, and in a three-way mix on annual grass species in the field (MSU-1) in 2020. Experiment biomass consisted mainly of annual grass species.

	Annual grass[a] control								Biomass[e]		
	14 DAT				42 DAT				42 DAT		
Treatment[b]	Expected[c]	Observed[d]			Expected	Observed			Expected[f]	Observed	
	%								% reduction		
glyphosate + AMS		100	a[g]			49	a			59	a
glufosinate + AMS		39	c			13	de			29	b
dicamba + CAR + DRA		0	e			0	e			0	c
glyphosate + glufosinate + AMS	100	95	a		55	35	b		71	55	ab (-)
dicamba + glyphosate + CAR + DRA	100	95	a		49	30	bc		42	43	ab
dicamba + glufosinate + MON	39	21	d (-)		13	4	e (-)		8	0	c
dicamba + glyphosate + glufosinate + MON	100	84	b (-)		55	19	cd (-)		58	32	ab (-)

[a] Annual grass species consisted of barnyardgrass, giant foxtail, and yellow foxtail.

[b] See table 3.2 for herbicide and adjuvant product information and rates.

[c] Expected control values were calculated using Gowing (1960) equation $E = A + (\frac{B(100-A)}{100})$ for paired combinations and Colby (1967) equation $E = A + B + C - \left(\frac{AB+AC+BC}{100}\right) + \left(\frac{ABC}{10{,}000}\right)$ for three-way mixtures.

[d] Antagonisms or synergisms are denoted by (-) or (+), respectively; if no notation is present, mixtures were additive at α ≤ 0.05.

[e] Experiment biomass consisted mainly of annual grass species.

[f] Expected biomass reductions were calculated using Colby (1967) equation $E = \frac{AB}{100} \quad OR \quad E = \frac{ABC}{10{,}000}$

[g] Means followed by the same letter are not statistically different at α ≤ 0.05.

Table 3.8. Activity of dicamba, glyphosate, and glufosinate applied alone, in paired combinations, and in a three-way mix on glyphosate-resistant horseweed in the field (MSU-2). Data were combined over years.

	Horseweed control								Horseweed biomass		
	14 DAT				42 DAT				42 DAT		
Treatment[a]	Expected[b]	Observed[c]			Expected	Observed			Expected[d]	Observed	
	%								% reduction		
glyphosate + AMS		12	e[e]			9	d			26	c
glufosinate + AMS		92	a			39	c			62	b
dicamba + CAR + DRA		62	d			90	ab			86	a
glyphosate + glufosinate + AMS	93	85	b		43	28	c (-)		71	51	b (-)
dicamba + glyphosate + CAR + DRA	67	71	c		91	96	a (+)		89	81	a
dicamba + glufosinate + MON	97	86	b (-)		93	82	b (-)		95	77	a (-)
dicamba + glyphosate + glufosinate + MON	97	83	b (-)		94	83	ab (-)		96	79	a (-)

[a] See table 3.2 for herbicide and adjuvant product information and rates.

[b] Expected control values were calculated using Gowing (1960) equation $E = A + (\frac{B(100-A)}{100})$ for paired combinations and Colby (1967) equation $E = A + B + C - \left(\frac{AB+AC+BC}{100}\right) + \left(\frac{ABC}{10{,}000}\right)$ for three-way mixtures.

[c] Antagonisms or synergisms are denoted by (-) or (+), respectively; if no notation is present, mixtures were additive at $\alpha \leq 0.05$.

[d] Expected biomass reductions were calculated using Colby (1967) equation $E = \frac{AB}{100} \quad OR \quad E = \frac{ABC}{10{,}000}$

[e] Means followed by the same letter are not statistically different at $\alpha \leq 0.05$.

Table 3.9. Activity of dicamba, glyphosate, and glufosinate applied alone, in paired combinations, and in a three-way mix on glyphosate-resistant waterhemp in the field (ISB-1). Data were combined over years.

	14 DAT				42 DAT			
	Control		Biomass		Control		Biomass	
Treatment[a]	Expected[b]	Observed[c]	Expected[d]	Observed	Expected	Observed	Expected	Observed
	——% ——		—— % reduction ——					
glyphosate + AMS		21 c[e]		56 d		27 d		41 b
glufosinate + AMS		90 a		92 a		69 c		83 a
dicamba + CAR + DRA		79 b		71 cd		86 b		89 a
glyphosate + glufosinate + AMS	92	88 a (-)	94	92 a	77	63 c (-)	85	87 a
dicamba + glyphosate + CAR + DRA	84	91 a	82	77 bc	90	96 a (+)	92	99 a (+)
dicamba + glufosinate + MON	98	90 a (-)	97	84 a-c (-)	95	88 ab (-)	97	95 a
dicamba + glyphosate + glufosinate + MON	98	90 a (-)	97	88 ab (-)	96	87 ab (-)	98	93 a

[a] See table 3.2 for herbicide and adjuvant product information and rates.

[b] Expected control values were calculated using Gowing (1960) equation $E = A + (\frac{B(100-A)}{100})$ for paired combinations and Colby (1967) equation $E = A + B + C - \left(\frac{AB+AC+BC}{100}\right) + \left(\frac{ABC}{10{,}000}\right)$ for three-way mixtures.

[c] Antagonisms or synergisms are denoted by (-) or (+), respectively. if no notation is present, mixtures were additive at $\alpha \leq 0.05$.

[d] Expected biomass reductions were calculated using Colby (1967) equation $E = \frac{AB}{100}\ \ OR\ \ E = \frac{ABC}{10{,}000}$

[e] Means followed by the same letter are not statistically different at $\alpha \leq 0.05$.

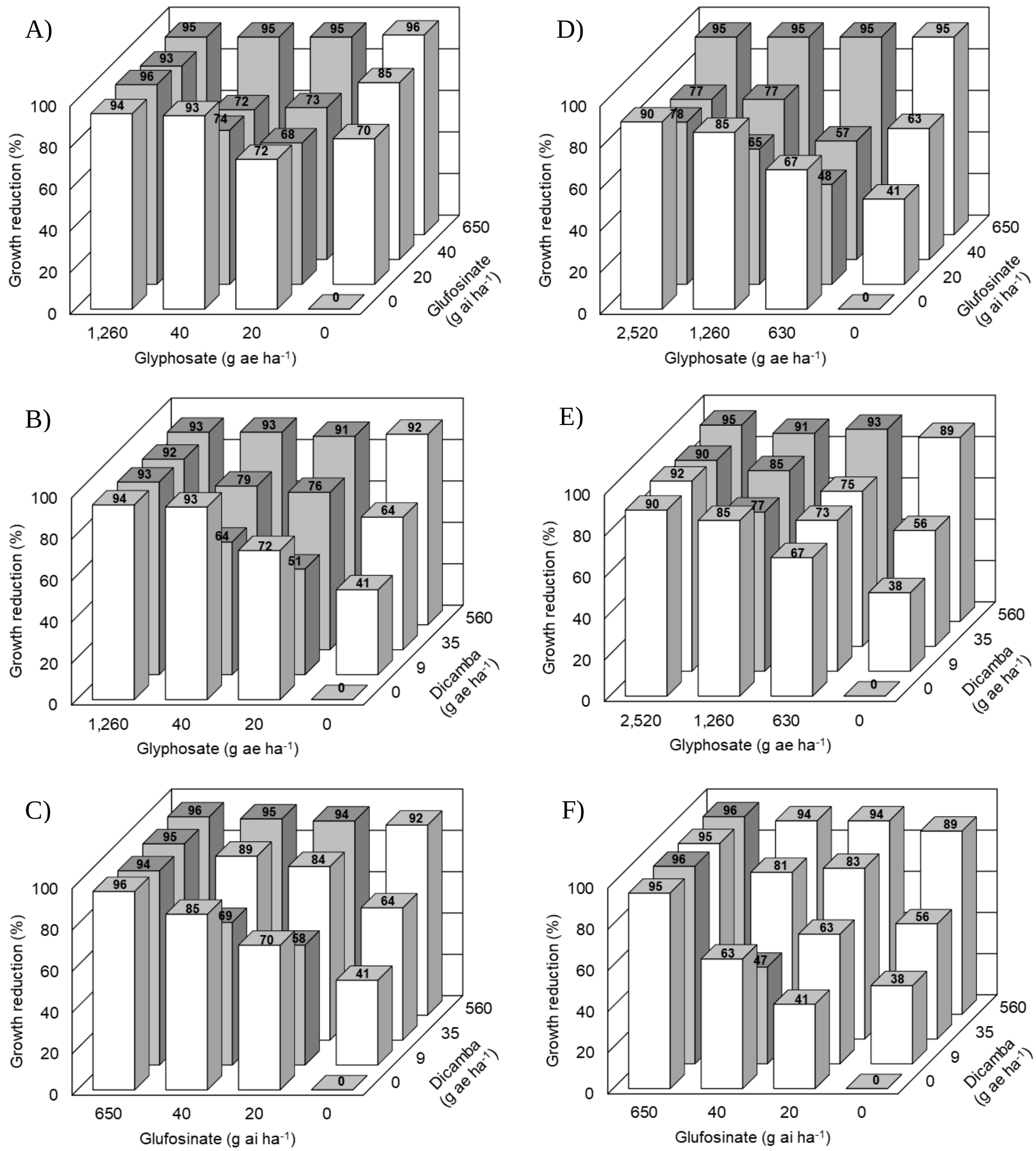

Figure 3.1. A-C: Reduction in glyphosate-susceptible Palmer amaranth dry weight in the greenhouse 14 DAT with combinations of (A) glyphosate + glufosinate ($LSD_{0.05}$ = 9), (B) dicamba + glyphosate ($LSD_{0.05}$ = 10), and (C) dicamba + glufosinate ($LSD_{0.05}$ = 9). **D-F:** Reduction in glyphosate-resistant Palmer amaranth dry weight in the greenhouse 14 DAT with combinations of (D) glyphosate + glufosinate ($LSD_{0.05}$ = 13), (E) dicamba + glyphosate ($LSD_{0.05}$ = 10), and (F) dicamba + glufosinate ($LSD_{0.05}$ = 11). White bars indicate an additive response, shaded bars represent an antagonistic response, and black bars represent a synergistic response.

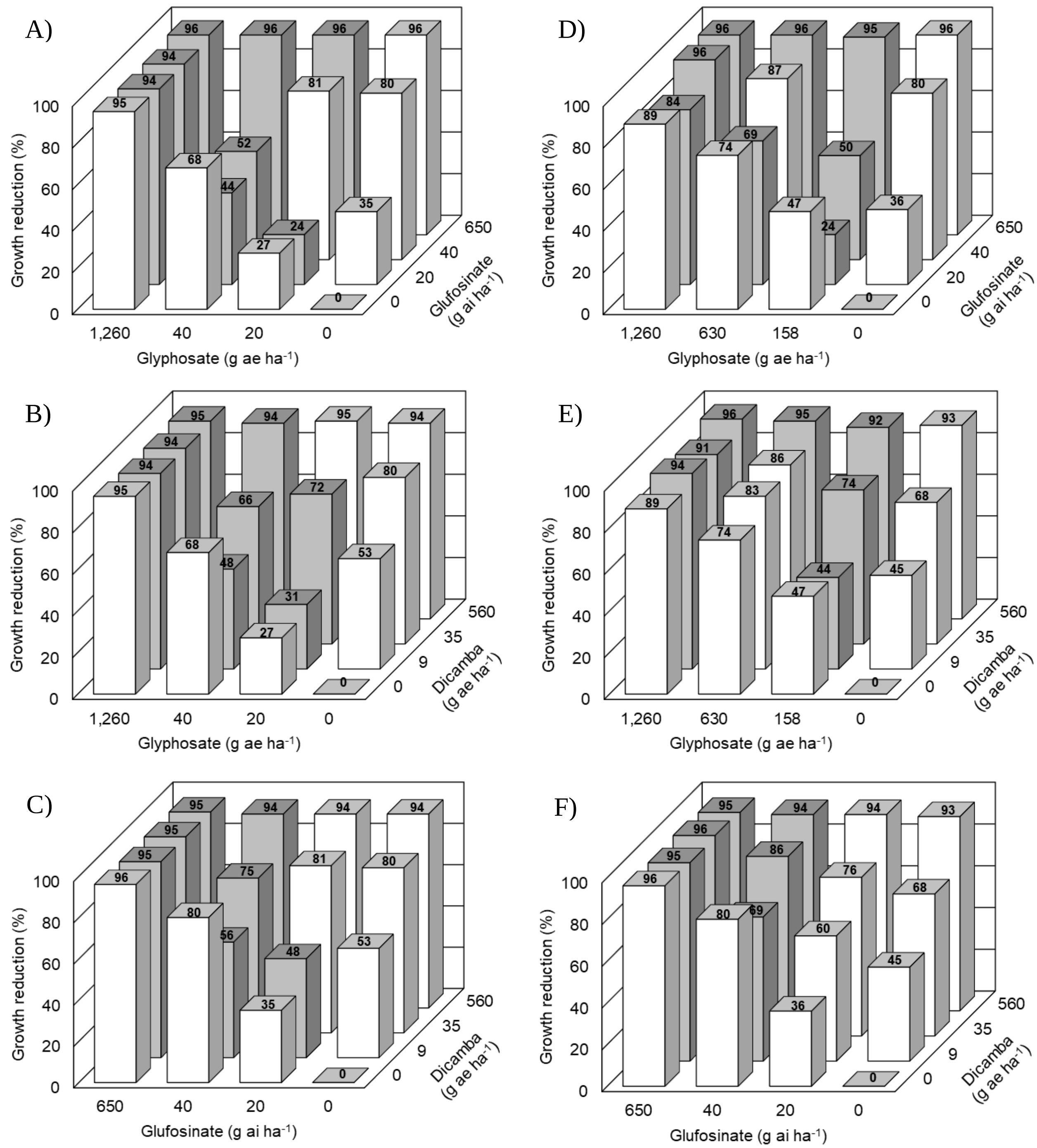

Figure 3.2. A-C: Reduction in glyphosate-susceptible waterhemp dry weight in the greenhouse 14 DAT with combinations of (A) glyphosate + glufosinate ($LSD_{0.05}$ = 15), (B) dicamba + glyphosate ($LSD_{0.05}$ = 12), and (C) dicamba + glufosinate ($LSD_{0.05}$ = 11). **D-F:** Reduction in glyphosate-resistant waterhemp dry weight in the greenhouse 14 DAT with combinations of (D) glyphosate + glufosinate ($LSD_{0.05}$ = 14), (E) dicamba + glyphosate ($LSD_{0.05}$ = 10), and (F) dicamba + glufosinate ($LSD_{0.05}$ = 8). White bars indicate an additive response, shaded bars represent an antagonistic response, and black bars represent a synergistic response.

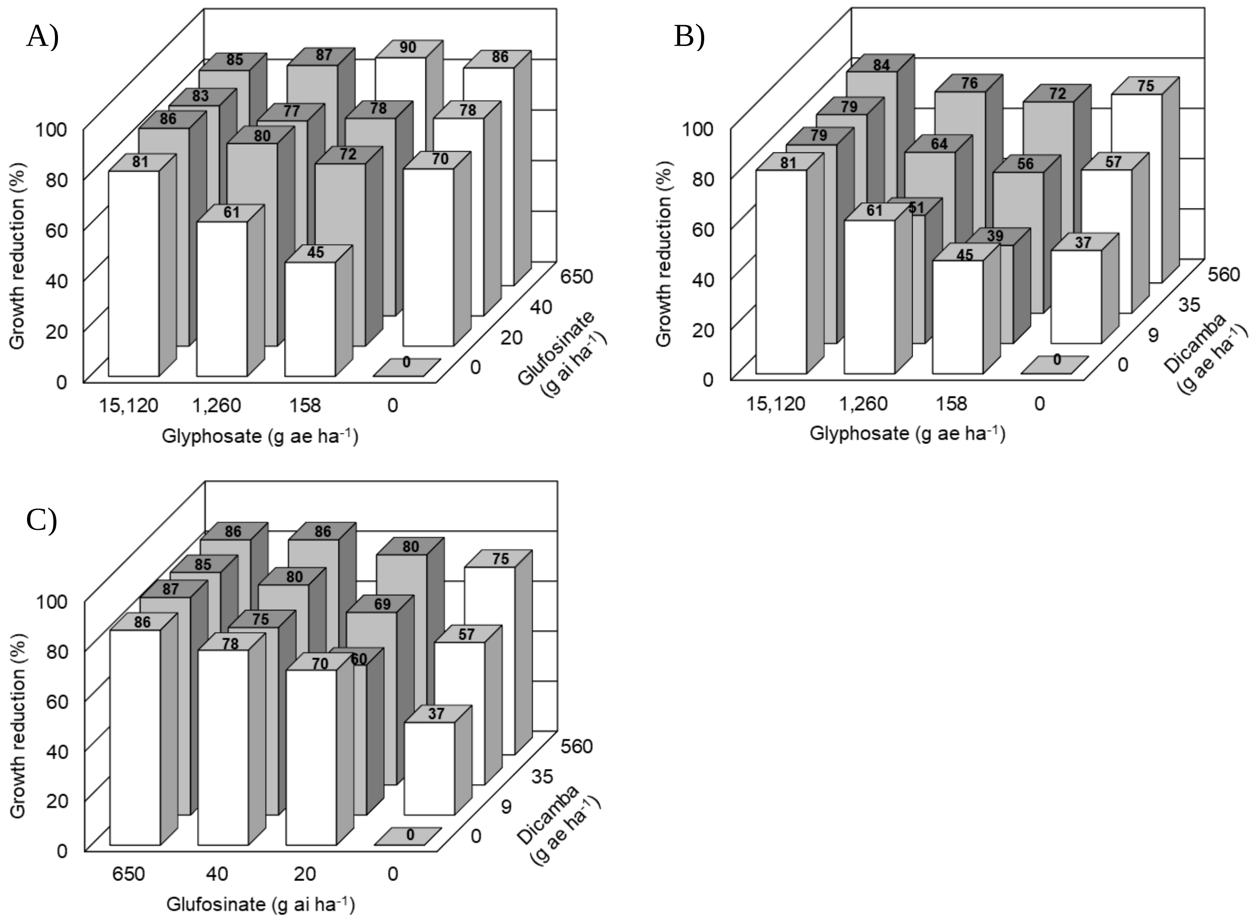

Figure 3.3. A-C: Reduction in glyphosate-resistant horseweed dry weight in the greenhouse 14 DAT with combinations of (A) glyphosate + glufosinate ($LSD_{0.05}$ = 8), (B) dicamba + glyphosate ($LSD_{0.05}$ = 11), and (C) dicamba + glufosinate ($LSD_{0.05}$ = 10). White bars indicate an additive response, shaded bars represent an antagonistic response, and black bars represent a synergistic response.

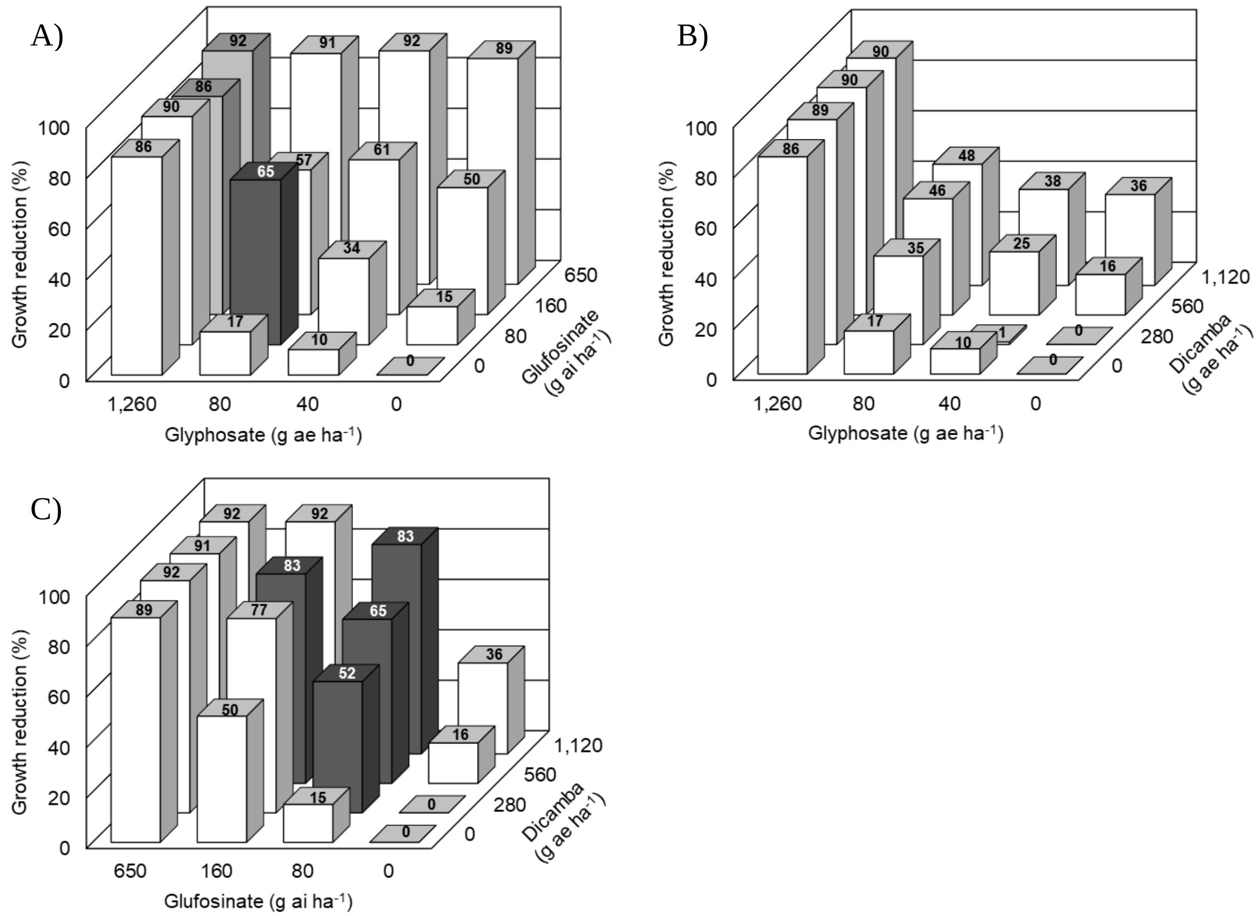

Figure 3.4. A-C: Reduction in barnyardgrass dry weight in the greenhouse 14 DAT with combinations of (A) glyphosate + glufosinate ($LSD_{0.05}$ = 21), (B) dicamba + glyphosate ($LSD_{0.05}$ = 18), and (C) dicamba + glufosinate ($LSD_{0.05}$ = 19). White bars indicate an additive response, shaded bars represent an antagonistic response, and black bars represent a synergistic response.

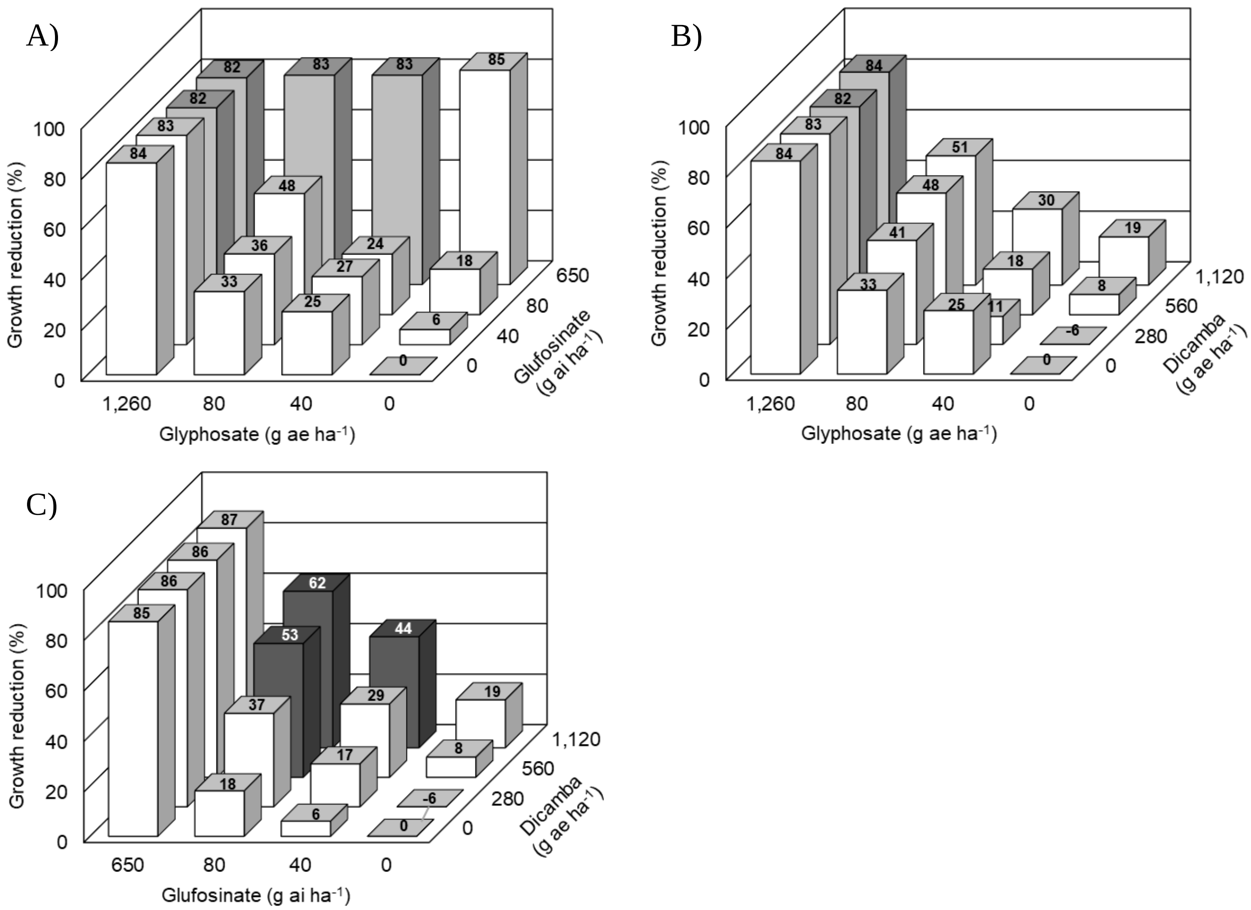

Figure 3.5. A-C: Reduction in yellow foxtail dry weight in the greenhouse 14 DAT with combinations of (A) glyphosate + glufosinate ($LSD_{0.05}$ = 14), (B) dicamba + glyphosate ($LSD_{0.05}$ = 16), and (C) dicamba + glufosinate ($LSD_{0.05}$ = 15). White bars indicate an additive response, shaded bars represent an antagonistic response, and black bars represent a synergistic response.

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