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SPECIAL ISSUE

REVIEW ARTICLE

Seventy-five years of synthetic herbicide use in agriculture: Will there be 100?

Herbicide mixtures: interactions and modeling

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Abstract: Mixing herbicides is a common practice in agriculture, to optimize farm management practices, widen the weed control spectrum, enhance application efficiency, and manage herbicide resistance. Interactions between herbicides can occur within the spray tank, on the leaf surface, and/or inside the plant, resulting in physicochemical and physiological interactions. Additive, synergistic, or antagonistic effects can result from these interactions. Given the range in plant response and the potential for negative and positive outcomes for weed management, predicting herbicide interactions before using mixtures would be of great value. Therefore, the physiological responses in the literature can help to ensure efficient weed control and avoid unwanted effects in the field. This review compiles information on physiological and physicochemical interactions between herbicides, addressing the most known cases of synergism, antagonism, and additivity, as well as their physiological bases, and the methods for evaluating herbicide interactions. Reference models for herbicide interactions have been reported and they usually interfere interpretation of the mixture effect. Antagonistic interactions can increase the evolution of weed resistance by favoring the survival of individuals exposed to the herbicide. Physicochemical incompatibility in the spray tank usually causes herbicide antagonism, whereas both synergism and antagonism can result from increased or decreased uptake/translocation and from physiological changes in the plant.

Keywords: Chemical weed control; synergism; antagonism; physiological basis

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1. Introduction

Weeds are considered the leading cause of crop yield losses worldwide, affecting food, fiber, and biofuel production. In addition, weeds can impact the quality of the final product and increase production costs, with severe economic impact on agricultural activities (Chauhan, 2020; Soltani et al., 2017). Therefore, adopting weed management practices is essential for maximizing crop yield and quality.

Chemical control is one of the most widely adopted methods to manage weeds in agriculture, primarily because it is easy to use, fast action, and efficiency (Dan et al., 2011). However, the absence of new molecules with new modes of action (MoA) in the last decades, along with the reliance on herbicides promoted the evolution of weed resistance to herbicides (Chauhan, 2020; Gaines et al., 2020). To date, 263 weed species resistant to 21 of the 31 MoA are reported worldwide, adding up to 504 unique resistance cases (Heap, 2022). With few effective herbicides available, the need to use alternative active ingredients, and increased weed control costs, diverse management strategies are needed to prevent resistance evolution.

Weed resistance management includes alternative practices that reduce the number and frequency of herbicide applications (Beckie, Harker, 2017; Norsworthy et al., 2012). Herbicides are the main selection agents of resistant plants, with reports of resistance evolution within three generations of susceptible rigid ryegrass (*Lolium rigidum* Gaud.) exposure to diclofop-methyl (acetyl-CoA carboxylase -inhibiting - ACCase, group 1) in Australia (Manalil et al., 2011; Neve, Powles, 2005). Using herbicides with different MoA is an important strategy to prevent herbicide resistance (Bianchi et al., 2020). One approach to use multiple MoAs is tank-mixing the herbicides.

The use of herbicide mixtures with different MoA at recommended rates reduces the chances of resistant individuals surviving and reproducing, especially when target-site resistance mechanisms are involved (Norsworthy et al., 2012). Furthermore, slower resistance evolution is expected when adopting herbicide mixtures compared to herbicide rotations (Busi and Beckie, 2021). In rigid ryegrass from Australia, only 8% of the studied biotypes showed resistance to trifluralin when mixed with prosulfocarb, while 36% and 51% were resistant to prosulfocarb or trifluralin, respectively. This suggests that herbicide mixtures reduce the resistance frequency compared to

individual applications and, thus, can delay the evolution of herbicide resistance (Busi, Beckie, 2021).

This review compiles information on the physiological and physicochemical interactions between herbicides, addressing the well-known cases of synergism, antagonism, and additivity between herbicides, their physiological basis, and the methods for evaluating interactions between them. The focus is entirely on how herbicides interact, as this review will not discuss how herbicide efficacy is impacted by different water quality factors, fertilizers, or other pesticides used in the mixture.

2. Herbicide interactions: synergism, antagonism, and additivity

The herbicides in the spray tank must be applied on the weeds and undergo the subsequent steps to exhibit their phytotoxic action. After deposition and retention on the target weed, the active ingredient must be absorbed by the plant leaf and translocate to its site of action, where it must accumulate in sufficient quantities to cause plant death (Cobb, Reade, 2010; Délye et al., 2013).

Herbicides may undergo physicochemical and physiological interactions, resulting in additive, synergistic, or antagonistic effects on weed control (Sørensen et al., 2007; Streibig et al., 1998). Additive effects in mixtures of two or more herbicides occur when the phytotoxicity observed in the biological target following herbicide application is not different from the expected phytotoxicity of the herbicides applied on their own (Rustom et al., 2019; Sørensen et al., 2007). However, in some situations, the interaction between herbicides results in significant changes in weed control responses, decreasing or increasing effectiveness. Antagonism is undesirable interaction in terms of weed control since the observed control is lower compared with the expected control of the herbicides applied alone, and in most cases, higher doses of herbicides are needed for satisfactory levels of control (Meyer et al., 2021; Zhang et al., 1995).

On the other hand, synergy is a term describing any type of interaction or cooperation that produces an outcome greater than the simple sum of its parts. The term is derived from the Greek word synergos, meaning "working together". Examples of this phenomenon are common in biological systems, which has led to the Synergism Hypothesis. This hypothesis states that synergistic interactions often provide functional survival and reproduction advantages favored by natural selection. With respect to the synergy between chemicals used in agriculture, the principles affecting these interactions are similar to that described for some of the drug synergisms (Jia et al., 2009): (1) One chemical may increase the bioavailability of the other chemical; (2) One chemical increases the potency of the other chemical; (3) One chemical prevents or delays the degradation of the other chemical; (4) Two chemicals act on the same physiological process but with different mechanisms; or (5) Two chemicals act on the same biosynthetic pathway but inhibit different targets.

The effects brought about by the interactions between herbicides are complex and cannot be totally predictable, even generating doubts about the physiological processes involved. Furthermore, physiological interactions can vary depending on the target plant species, the stage of weed growth, the doses used, the environment, and the physicochemical and physiological characteristics of the herbicides (Hammerton, 1967; Zhang et al., 1995). A study of the interactions between different mixtures of glufosinate and clethodim demonstrated that the antagonism depended on the doses used and the target weed species (Meyer et al., 2021). Mixtures of atrazine with herbicide inhibitors of different sites in the carotenoid biosynthesis pathway resulted in a variety of physiological responses: synergistic effects were observed in the control of red morningglory (*Ipomoea coccinea* L.) for mixtures of atrazine with clomazone, mesotrione, and norflurazon, while the same mixtures showed antagonism, synergism, and additive effects, respectively, to common cocklebur (*Xanthium strumarium* L.), at all doses (Armel et al., 2007).

The type of interaction and, in turn, the resulting efficacy on weeds can be affected by physicochemical characteristics such as pKa and Kow, as well as physiological characteristics like the mode of action and metabolism. As a result of these characteristics, the interactions between herbicides can result in changes in the rate of absorption, translocation, and metabolism of one herbicide due to the presence of another(s), resulting in an increase or decrease in the amount of biologically active herbicide at the site of action, as well as changes at specific points in the herbicide's action pathway (Green, 1989; Zhang et al., 1995).

Antagonistic interactions can also be attributed to the increased metabolism of one herbicide due to the presence of another herbicide in the mixture. Accelerated metabolism of herbicides into non-toxic molecules is considered a resistance mechanism in many weeds and is typical for herbicides in the aryloxyphenoxypropionate chemical group (Takano et al., 2020a; Yu, Powles, 2014). A large body of studies has confirmed the involvement of P450 monooxygenase enzymes in the accelerated metabolism of a wide array of herbicides in various weeds (Fernández et al., 2016; Nandula et al., 2019) and even in crops such as wheat (*Triticum aestivum* L.) (Zimmerlin, Durst, 1990). Thus, the antagonism in the mixture of 2,4-D with diclofop-methyl, for example, in the control of rigid ryegrass, may be due to the expression of P450 genes, which encode the enzymes responsible for the herbicide degradation. This reduces the concentration of the toxic form of the pesticide is reduced to the point that it can no longer inhibit the plants' metabolic pathways (Han et al., 2013).

Given the range in plant responses and the potential for both negative and positive outcomes for weed management, predicting herbicide interactions before using mixtures

would be of great value to weed managers. However, little progress has been made over the last 50 years in our ability to accurately predict new herbicide interactions, likely a function of the diversity of experimental methods, physiochemical, biochemical, plant, and environmental factors that determine these interactions. The interaction of picloram and 2,4-D was classified as antagonistic, synergistic, and additive on field bindweed (*Convolvulus arvensis* L.), depending on the number of days after treatment the plants were evaluated (Agbakoba, Goodin, 1970). In the same experiment, the interaction of these auxinic herbicides was antagonistic when applied at close to field use rates and synergistic when applied at 1/100x and 1/1,000x of field use rates. The mixture of bentazon and acifluorfen on redroot pigweed (*Amaranthus retroflexus* L.) was described as antagonistic under glasshouse conditions, yet the same experiment conducted with the plants outside altered the plant response to synergism (Figure 1; Sorensen et al., 1987). In the glasshouse, these same herbicide combinations resulted in synergistic responses on common lambsquarters (*Chenopodium album* L.) when no spray adjuvant was included but produced only additive responses when the herbicides were applied with crop oil concentrate (Figure 1; Sorensen et al., 1987).

Zhang et al. (1995) analyzed a database containing 479 published herbicide interactions to identify any trends that could inform the mechanism of herbicide interactions. The location of herbicide absorption on the plant, the extent of herbicide translocation, the similarity of the herbicide mechanisms or modes of action, nor the life history of the plant were effective predictors of how herbicide mixtures would interact (Zhang et al., 1995). The only reliable finding of this study was that herbicide antagonism was documented much more frequently than synergism, and the target weed

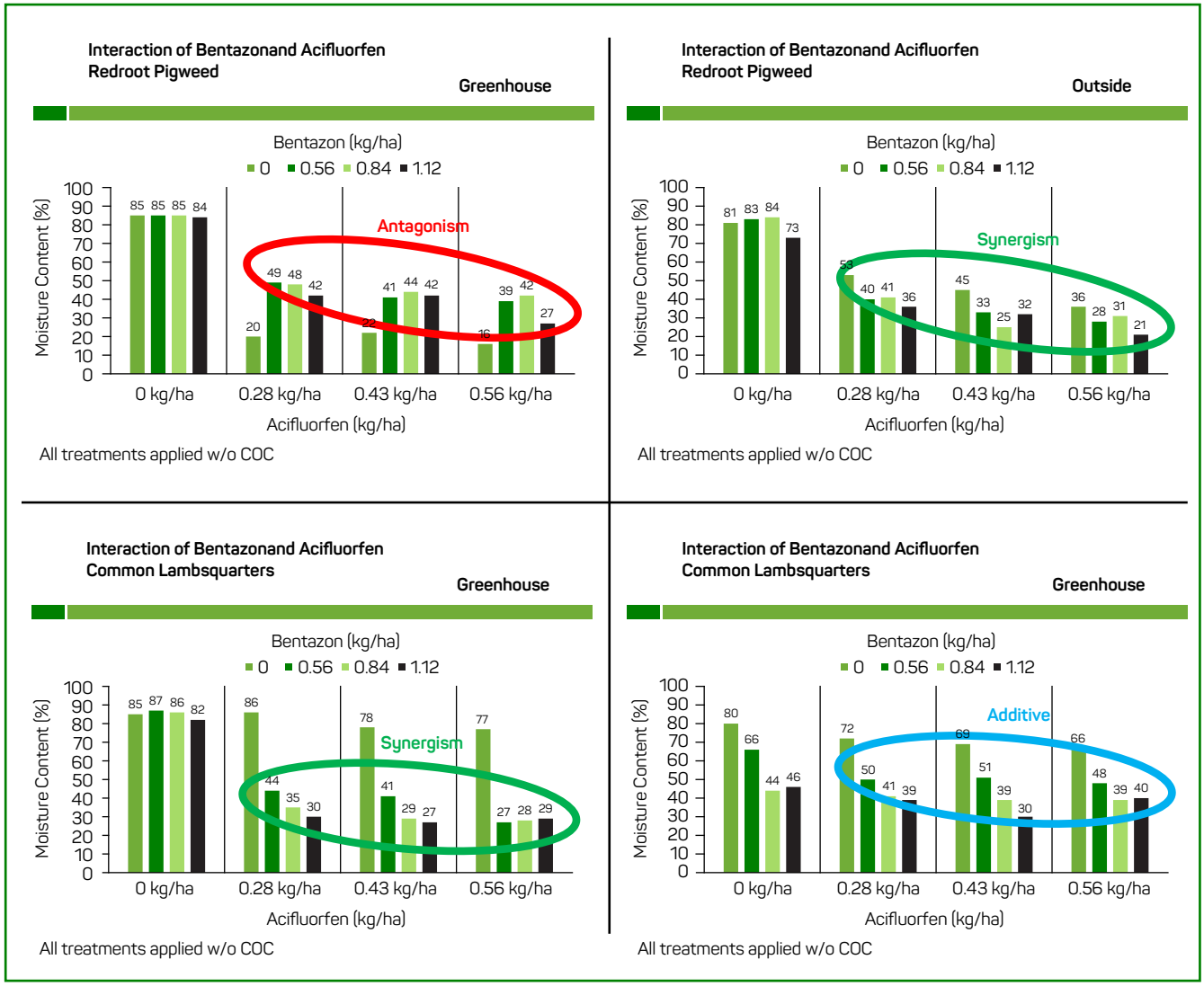

Figure 1 - Influence of plant growing conditions and adjuvant on the interaction of bentazon and acifluorfen. Adapted from Sorensen et al. (1987) - Used under permission - License number = 5430850159759). Redroot pigweed: *Amaranthus retroflexus* L. Common lambsquarters: *Chenopodium album* L.

species more commonly associated with the antagonistic interactions were monocots compared to dicots. The most logical explanation for this observation is: 1) antagonism is easily observed and problematic commercially that requires research to develop recommendations to avoid reductions in weed control, and 2) group 1 herbicides were important postemergence herbicides used in several cropping systems and had to be applied with broadleaf herbicides to achieve control of the full weed spectrum. Unfortunately, the group 1 herbicides can be antagonized by several other herbicide MoA groups, resulting in numerous studies being conducted to resolve the antagonism.

Identifying which herbicide in the mixture is acting as the antagonist or synergist is not always possible from whole-plant experiments. The antagonist is assumed to be the herbicide that contributes less herbicide efficacy when applied alone. However, this may be difficult to determine if both herbicides have relatively similar levels of herbicide activity on the particular plant species. The synergist can be even more challenging to identify because the enhancement in overall herbicide efficacy may originate from an herbicide with relatively high or low levels of herbicide activity. In fact, true synergists may not have any phytotoxicity at all on the target species. Thus, more in-depth research regarding herbicide absorption, translocation, metabolism, or enzyme inhibition for one or both herbicides in the mixture may be necessary to designate an herbicide antagonist or synergist conclusively.

The following summarizes the most common observations of herbicide synergism and antagonism in the literature. This discussion is not intended to serve as a complete list of herbicide interactions reported. Furthermore, discussing a specific type of herbicide interaction (e.g., synergism) for two herbicide mode of action groups does not preclude the possibility that other mixture interactions may occur for that same combination under different experimental conditions. In other words, just because two herbicides are reported below to interact synergistically does not negate the possibility that these herbicides may also result in additive or antagonistic interactions.

2.1 Examples of synergistic herbicide interactions

Synergistic herbicide combinations reported by herbicide and plant species, which will be addressed in the next sections and some examples are provided in Table 1.

2.1.1 *Group 2 herbicides*

A common synergistic interaction is the combination of group 2 herbicides. The addition of imazapyr to another imidazolinone herbicide, imazaquin or imazethapyr, increased control of pitted morningglory (*Ipomoea lacunosa* L.) and johnsongrass [*Sorghum halepense* (L.) Pers.] by up to 21% (Riley, Shaw, 1988). The synergistic interaction was independent of temperature and relative humidity (Kent et al., 1991). Likewise, the combination of a half rate (70 g ai ha^{-1}) of imazaquin was synergistic with imazethapyr to increase control of johnsongrass and pitted morningglory by up to 30% (Riley, Shaw, 1988). Similar enhancements in herbicide efficacy were observed for imazapic applied with imazaquin and imazethapyr on both monocot and dicot species, along with an increase in soybean injury (Shaw, Wixson, 1991). Within the sulfonylurea chemical family, combinations of nicosulfuron and rimsulfuron were identified as synergistic for control of smooth crabgrass [*Digitaria ischaemum* (Schreb.) Muhl.], additive on corn (*Zea mays* L.), and antagonistic on soybean (Mekki, Leroux, 1994).

The group 14 herbicides have frequently been applied with group 2 herbicides to broaden the spectrum of weed control in postemergence applications in soybean for several decades. The type of herbicide interaction that results from combinations involving group 14 herbicides is quite variable. Imazaquin or chlorimuron applied with acifluorfen, fomesafen, or lactofen produced some synergistic interactions for control of prickly sida (*Sida spinosa* L.) and pitted morningglory (Wesley, Shaw, 1992). However, these same herbicide mixtures resulted in additive or antagonistic responses on the same weed species depending on the dose of each herbicide applied and the weed growth stage.

2.1.2 *Group 4 herbicides*

The auxin mimic (group 4) herbicides is the first synthetic, selective herbicides having a significant commercial impact on weed management and remain prominent herbicides for weed control in numerous sites. Early research to improve weed control would frequently combine herbicides within this group because other modern herbicides were not yet available. Applying picloram with 2,4-D amine, mecoprop, and 2,4,5-T resulted in synergism for controlling annual and perennial dicot species (Agbakoba, Goodin, 1970; Bovey et al., 1968; Hamill et al., 1972; Scifres, 1972).

The use of group 4 herbicides is also synergistic when applied in a mixture with glyphosate (group 9). The combination of a low dose of 2,4-DB (45 g ae ha^{-1}) with glyphosate increased the control of palmleaf morningglory (*Ipomoea wrightii* Gray) from 13% for either herbicide alone to 99% for the mixture (Wehtje, Walker, 1997). This combination was synergistic at several dose combinations, at multiple weed growth stages, and across three other species of morningglory. Similar research found glyphosate applied with either 2,4-D or dicamba resulted in synergistic activity on emerging shoots of field bindweed, a perennial species, with the interaction consistent on the amount of shoot regrowth following application (Flint, Barrett, 1989a). The increased herbicidal activity was attributed to greater foliar uptake and accumulation in the roots for 2,4-D and dicamba when applied with glyphosate.

Table 1 - Partial list of synergistic herbicide combinations reported by herbicide and plant species. An herbicide combination resulting in a synergistic interaction does not suggest the interaction remains constant across herbicide dose, plant species, or experimental conditions. For information on plant species, please refer to the individual reference articles

Herbicide 'A'		Herbicide 'B'		Reference
SOA Group	Herbicide	SOA Group	Herbicide	
1	Sethoxydim	1	Fluazifop-butyl	Harker and O'Sullivan, 1991
1	Fluazifop-butyl	14	Acifluorfen	Minton et al., 1989b
		14	Fomesafen	Minton et al., 1989b
	Haloxyfop-methyl	14	Acifluorfen	Minton et al., 1989b
		14	Fomesafen	Minton et al., 1989b
	Quizalofop-ethyl	14	Acifluorfen	Minton et al., 1989b
	Sethoxydim	14	Acifluorfen	Minton et al., 1989b
2	Imazapyr	2	Imazaquin	Riley and Shaw, 1988
		2	Imazethapyr	Riley and Shaw, 1988
		2	Metsulfuron	Kudsk and Mathiassen, 2004
	Imazaquin	2	Imazethapyr	Riley and Shaw, 1988
	Imazapic	2	Imazaquin	Riley and Shaw, 1989, 1988
		2	Imazethapyr	Riley and Shaw, 1989, 1988
	Nicosulfuron	2	Rimsulfuron	Mekki and Leroux, 1994
2	Ethametsulfuron-methyl	4	Clopyralid	Blackshaw, 1989
	Imazamox	4	Dicamba	Kelley et al., 2005
	Imazethapyr	4	Dicamba	Kelley et al., 2005
	Metsulfuron	4	MCPA	Hollaway et al., 1996
2	Chlorimuron	9	Glyphosate	Norris et al., 2001
	Cloransulam	9	Glyphosate	Norris et al., 2001
	Imazapyr	9	Glyphosate	Kudsk and Mathiassen, 2004
	Imazaquin	9	Glyphosate	Hydrick and Shaw, 1994; Norris et al., 2001
	Imazethapyr	9	Glyphosate	Norris et al., 2001; Starke and Oliver, 1998
	Metsulfuron	9	Glyphosate	Kudsk and Mathiassen, 2004
2	Imazapyr	10	Glufosinate	Kudsk and Mathiassen, 2004
	Metsulfuron	10	Glufosinate	Kudsk and Mathiassen, 2004
2	Chlorimuron	14	Acifluorfen	Wesley and Shaw, 1992
		14	Fomesafen	Wesley and Shaw, 1992
		14	Lactofen	Wesley and Shaw, 1992
	Imazaquin	14	Acifluorfen	Wesley and Shaw, 1992
		14	Fomesafen	Wesley and Shaw, 1992
		14	Lactofen	Wesley and Shaw, 1992
4	Picloram	4	2,4,5-T	Bovey et al., 1968; Scifres, 1972
		4	2,4-D amine	Agbakoba and Goodin, 1970
		4	Mecoprop	Hamill et al., 1972
4	2,4-D	5	Ametryne	Diem, Davis, 1974
4	2,4-DB	6	Pyridate	Hicks et al., 1998
4	2,4-D	9	Glyphosate	Flint and Barrett, 1989a
	2,4-DB	9	Glyphosate	Wehtje and Walker, 1997
	Dicamba	9	Glyphosate	Flint and Barrett, 1989a; Kelley et al., 2005

Continue

Table 1 (continued).

Herbicide 'A'		Herbicide 'B'		Reference
SOA Group	Herbicide	SOA Group	Herbicide	
	Fluroxypyr	9	Glyphosate	Chorbadjian and Kogan, 2002
4	Dicamba	14	Fomesafen	Kelley et al., 2005
4	2,4-DB	22	Paraquat	Wehtje et al., 1992a
	Chloramben	22	Paraquat	Wehtje et al., 1992c
	Picloram	22	Paraquat	Bovey et al., 1968
5	Propanil	3	Pendimethalin	Norsworthy et al., 1999
5	Desmedipham	5	Phenmedipham	Anderson and Arnold, 1984
	Methabenz thiazuron	5	Simazine	Faust et al., 1993
5	Propanil	10	Glufosinate	Lanclos et al., 2002
5	Propanil	15	Anilofos	Norsworthy et al., 1999
	Methabenz thiazuron	15	Metazachlor	Faust et al., 1993
5	Atrazine	27	Mesotrione	Abendroth et al., 2006
	Metribuzin	27	Mesotrione	Abendroth et al., 2006
6	Bentazon	14	Acifluorfen	Sorensen et al., 1987
6	Bentazon	22	Paraquat	Wehtje et al., 1992b
6	Bromoxynil	27	Mesotrione	Abendroth et al., 2006
9	Glyphosate	14	Acifluorfen	Norris et al., 2001
		14	Flumiclorac	Norris et al., 2001
		14	Fomesafen	Norris et al., 2001
		14	Lactofen	Norris et al., 2001
12	Norflurazon	15	Metolachlor	Wehtje and Brecke, 2004
14	Lactofen	15	Alachlor	Moore and Banks, 1991
	Flumioxazin	15	Metolachlor	Wehtje and Brecke, 2004
14	Oxyfluorfen	22	Paraquat	Pritchard et al., 1980
15	Acetochlor	1	Fluazifop-butyl	Scott et al., 1998a
		1	Sethoxydim	Scott et al., 1998a
	Dimethenamid	1	Fluazifop-butyl	Scott et al., 1998a
		1	Sethoxydim	Scott et al., 1998a; 1998b
	Metolachlor	1	Fluazifop-butyl	Scott et al., 1998a
		1	Sethoxydim	Scott et al., 1998a
15	Acetochlor	2	Imazethapyr	Scott et al., 1998a
	Dimethenamid	2	Imazethapyr	Scott et al., 1998a, 1998b
	Metolachlor	2	Imazethapyr	Scott et al., 1998a
22	Diquat	0	MSMA	Bovey and Miller, 1968

The expected result of combining a fast-acting, non-systemic herbicide with a systemic herbicide would be an antagonism of the systemic herbicide due to a reduction in herbicide absorption or translocation from the rapid tissue necrosis caused by the other herbicide. However, combining a group 4 herbicide (2,4-DB) with paraquat (group 22) was synergistic for the control of smallflower morningglory [*Jacquemontia tamnifolia* (L.) Griseb], Florida beggarweed [*Desmodium tortuosum* (SW.) DC], and sicklepod (*Cassia obtusifolia* L.) (Wehtje et al., 1992a). Likewise, applying the group 4 herbicide chloramben with paraquat was synergistic on Florida beggarweed (Wehtje et al., 1992a). The synergy between picloram and paraquat was even evident in a perennial woody species (Bovey et al., 1968).

2.1.3 *Group 5 and 6 herbicides*

The foliar action of photosystem II inhibitors, especially on dicot species, and the soil residual activity

for some herbicides within this group have resulted in mixtures with herbicides from other mode of action groups to broaden the weed spectrum for emerged weeds and subsequent weed emergence events. Perhaps one of the most recognized and consistent synergistic herbicide interactions in research and commercial applications has been evident with mixtures of group 5/6 and 27 herbicides (HPPD inhibitors). Combining mesotrione with atrazine, bromoxynil, and metribuzin resulted in synergistic control of palmer amaranth, velvetleaf (*Abutilon theophrasti* Medic.), sunflower (*Helianthus annuus* L.), red morningglory, common cocklebur, and giant foxtail (*Setaria faberi* Herm.) (Abendroth et al., 2006; Armel et al., 2007). Furthermore, these interactions can occur when the group 5/6 herbicide is applied at 25% of the regular field use rate, which has allowed reduced herbicide rates in commercial applications. The physiological basis for the synergy between these herbicide mode of action groups is discussed in detail later in this review.

Complementary action may result from herbicide combinations from modes of action that target related biochemical pathways. In some instances, applying a photosystem II inhibitor (bentazon) with a photosystem I electron diverter (paraquat) resulted in synergism on smallflower morningglory (Wehtje et al., 1992b). However, with the range of herbicide doses, the response ranged from antagonistic to synergistic responses. The combination of paraquat with simazine or diuron was synergistic for the control of quackgrass [*Agropyron repens* (L.) Beauv.] (Putnam, Ries, 1967). Furthermore, Eubank et al. (2012) suggested that the combination of paraquat and metribuzin was synergistic on horseweed [*Conyza canadensis* (L.) Cronq.] that was resistant to paraquat.

2.1.4 *Group 15 herbicides*

Group 15 herbicides have traditionally been used for soil residual control of important grass and small-seeded broadleaf species, with no commercial use for control of emerged plants. Despite this, one study by Scott et al. (1998a) using both glasshouse and field experiments documented group 15 herbicides (dimethenamid, metolachlor, acetochlor) acting as synergists in combinations with group 1 herbicides (sethoxydim, fluazifop-P) and imazethapyr (group 2) on barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv], broadleaf signalgrass [*Brachiaria platyphylla* (Griseb.) Nash], johnsongrass, and red rice (*Oryza sativa* L.). An increase of up to 55% was observed for the group 15 herbicides with the group 1 herbicides and up to 49% for mixtures with imazethapyr (Scott et al., 1998a). Further research with dimethenamid demonstrated the synergism was influenced by the adjuvant system and dose of the fluazifop and sethoxydim, but neither dose nor adjuvant impacted the synergistic response with imazethapyr. Since commercial formulations of group 15 herbicides include oil-based solvents, a theory was formed that the improved efficacy of the group 1 herbicides with dimethenamid may have been an artifact of the product formulation serving as a penetrating oil similar to an adjuvant. However, an experiment using technical grade dimethenamid and the emulsifiable concentrate formulation blank concluded that the synergistic response with sethoxydim was caused by the herbicide molecule, not the formulation (Scott et al., 1998a).

2.2 Examples of antagonistic herbicide interactions

Herbicide antagonism is a problem for weed practitioners as it can result in failed weed control and the need for subsequent practices to remove escaped weeds. Unfortunately, from a weed management perspective, herbicide antagonism has been documented in the literature approximately twice as often than synergism (Zhang et al., 1995). Certainly, some scientists have concluded that true herbicide synergy is rare, and 70% of herbicide mixtures involving different herbicide sites of action result in antagonism (Cedergreen et al., 2007b). Two practical observations would also support these trends: 1) antagonistic herbicide mixtures are easier to identify than synergism in field situations since the result may be weed survival, and 2) documenting synergy requires an estimation of additivity for the mixture, whereas the simplest form of antagonism can be recognized as any weed control level less than either herbicide applied alone. The latter eliminates the need to use or understand any additivity model since those calculations would result in an expected value similar to or greater than the highest level of efficacy achieved with one of the herbicides alone.

2.2.1 *Group 1 herbicides*

The group 1 (ACCase-inhibiting) herbicides are the most commonly antagonized by other herbicides in the mixture. Reductions in the control of grass species have been reported when group 1 herbicides were combined with group 2, 4, 6, 9, and 14 herbicides. The desire to achieve broad-spectrum weed control most likely contributes to the high frequency of antagonism reported for group 1 graminicides and the requirement for tank mixing with herbicide to control dicot weeds. These herbicide combinations have been commonly used for postemergence weed control in several dicot and cereal crops.

Field and glasshouse applications of fluazifop-P, haloxyfop, quizalofop, sethoxydim, or fenoxaprop with the group 2 herbicides chlorimuron or imazaquin reduced control of red rice or barnyardgrass by up to 53% (Minton et al., 1989a; 1989b). Another example is the premix formulation of thifensulfuron and tribenuron antagonized clethodim on volunteer wheat but did not reduce the efficacy of quizalofop (Blackshaw et al., 2006). Overall, antagonism up to 70% on annual grass species has

been reported for serval group 1 herbicides applied with group 2 herbicides from the sulfonylurea and imidazolinone chemical families (Burke et al., 2002; Kammler et al., 2010; Liebl, Worsham, 1987; Liu et al., 1994; Minton et al., 1989a; 1989b; Nelson et al., 1998; O'Sullivan and Kirkland, 1984; Young et al., 1996; Zhang et al., 2005). The antagonism can be more pronounced at later weed growth stages, and, in some instances, applying the herbicides sequentially (20 min. apart) did not resolve the antagonism (Liebl, Worsham, 1987), suggesting the interaction is less likely physiochemical and more probable a plant physiological response. Liebl, Worsham (1987) inferred that applying the group 1 herbicide (diclofop-methyl) and group 2 herbicide (chlorsulfuron) in a manner that optimized individual herbicide efficacy, such as earlier plant growth stages or preemergence versus postemergence, can minimize the interaction and possibly revert to additivity. The use of a more aggressive adjuvant for foliar penetration (MSO) has been shown to resolve the antagonistic interaction between group 1 and 2 herbicides, but not for all herbicide actives and weed species (Kammler et al., 2010).

The group 4 herbicides are regarded as primarily postemergence herbicides for broadleaf weed control. Thus, combining these herbicides with the group 1 herbicides is logical and has historically been applied in a mixture for weed control in cereal crops. For instance, diclofop controls several important annual grass species in cereal crops. Combing 2,4-D amine with diclofop has been reported as antagonistic for wild oat control (*Avena fatua* L.) (Todd, Stobbe, 1980). Volunteer wheat can be problematic in rotational crops or fallow systems, requiring management tactics to be implemented. Applying clethodim and quizalofop with 2,4-D resulted in up to a 100% loss of herbicide efficacy on wheat (Blackshaw et al., 2006). The 2,4-D amine formulation was much more antagonistic than the ester formulation and increasing the dose of the group 1 herbicide reduced the level of antagonism. Blackshaw et al. (2006) concluded that 2,4-D ester, bromoxynil, or bromoxynil plus MCPA could be used with either clethodim or quizalofop commercially without concern for antagonizing control of volunteer wheat.

Barnyardgrass is one of the most problematic weeds in rice production globally (Silva et al., 2022). Applying triclopyr with fenoxaprop was antagonistic to the control of barnyardgrass (Zhang et al., 1995). Applications of fenoxaprop and fluazifop with 2,4-DB in soybean production reduced control of red rice and barnyardgrass by up to 20% (Minton et al., 1989a; 1989b). Similarly, applying 2,4-D with sethoxydim was antagonistic for control of shattercane [*Sorghum bicolor* (L.) Moench] (Young et al., 1996). Applying clethodim and either 2,4-D or dicamba simultaneously, yet in a separate boom system on the sprayer, eliminated the antagonism on Italian ryegrass (*Lolium multiflorum* Lam.) (Merritt et al., 2020).

The combination of group 1 herbicides with glyphosate has not been justified for the majority of glyphosate use since glyphosate has a high level of activity on monocots. However, the commercialization of glyphosate-resistant (GR) corn in the late 90s in the U.S. created the problem of volunteer GR corn in soybean production. Consequently, the use of a group 1 herbicide with glyphosate has been a common practice in GR soybean to control traditional weeds and GR volunteer corn. Combining these herbicide groups has produced inconsistent levels of herbicide efficacy on volunteer GR corn but has shown reductions by as much as 60% (Harre et al., 2020). The inclusion of 2,4-D or dicamba, enabled by current herbicide resistance traits in soybean, with glyphosate and clethodim magnifies the antagonism even further. An alternative would be using glufosinate-resistant soybean and use combinations of glufosinate with group 1 herbicides for control of the GR volunteer corn, since single applications of glufosinate alone is insufficient for control (Chahal, Jhala, 2015). However, applying glufosinate with full rates of group 1 herbicides (clethodim, quizalofop, fluazifop, fenoxaprop plus fluazifop, sethoxydim) antagonized the ACCase-inhibitors for control of GR volunteer corn (Chahal, Jhala, 2015).

As evolution of weeds with resistance to glyphosate continues, one of the first herbicides used as an alternative is glufosinate. Glufosinate has limited movement in plants which can be problematic for the control of grass species with the active shoot meristem in the whorl or base of the plant. Thus, applying a group 1 herbicide with glufosinate has been practiced to supplement grass efficacy. Burke et al. (2005) reported at least a 52-percentage point reduction in control of goosegrass [*Eleusine indica* (L.) Gaertn.] when glufosinate was applied in a mixture with clethodim and suggested the solution to the antagonism was to apply the mixture on goosegrass with no more than two tillers. Glufosinate has also been reported to antagonize clethodim, fluazifop, quizalofop, and sethoxydim on annual grasses and johnsongrass, and was not resolved by increasing the rate of the graminicide (Gardner et al., 2006). For both studies, the antagonism of the graminicide from glufosinate was avoided when the herbicides were applied separately by 3 to 7 days (Burke et al., 2005; Gardner et al., 2006).

The group 14 (PPO-inhibitor) herbicides are another group with foliar activity with a limited broadleaf weed spectrum that must be combined with other herbicides for complete weed control. Adding group 1 herbicides for foliar grass activity with group 14 herbicides has frequently been reported as antagonistic. Various mixtures of fenoxaprop, fluazifop, haloxyfop, quizalofop, and sethoxydim with acifluorfen, carfentrazone, flumiclorac, fluthiacet fomesafen, and lactofen have resulted in the antagonism on red rice, barnyardgrass, southern crabgrass [*Digitaria ciliaris* (Retz.) Koel.], Texas panicum (*Panicum texanum* Buckl.), large crabgrass [*Digitaria sanguinalis* (L.) Scop], and shattercane (Grichar, 1991; Minton et al., 1989a; 1989b; Young et al., 1996; Zhang et al., 2005).

Interestingly, the antagonism of group 1 herbicides includes herbicides that have limited efficacy on grass

species (e.g. herbicide groups 4, 6, 14), as well as herbicides that can have a high level of grass efficacy (e.g. group 2 herbicides, glyphosate, glufosinate). Successful practices to avoid or overcome the antagonistic interaction with group 1 herbicides includes applying the herbicides at a relatively small weed growth stage (Burke et al., 2005; Liebl, Worsham, 1987), applying the herbicide temporally separate by at least 3 days (Burke et al., 2005; Dotray et al., 1993), increasing the dose of the group 1 herbicide (Harre et al., 2020), applying the herbicides on smaller target weeds (Liebl, Worsham, 1987), and applying a more effective adjuvant to optimize the group 1 herbicide (Kammler et al., 2010). It is important to notice that the increase of herbicide rate can be done only up to the maximum labeled rate.

2.2.2 *Group 2 herbicides*

Tank mixtures containing multiple group 2 herbicides were discussed previously as resulting in synergism. However, these combinations can also produce antagonistic responses such as the mixture of imidazolinone herbicides imazapyr and imazaquin applied with imazethapyr (Riley, Shaw, 1989), imazapyr applied with metsulfuron (Kudsk, Mathiassen, 2004), and nicosulfuron applied with rimsulfuron (Mekki, Leroux, 1994). Since the level of efficacy may be similar for these herbicides on the target weed, the origin of the antagonism for these combinations can be difficult to distinguish without further experimentation on herbicide fate in the plant.

Management of broadleaf weeds in soybean can be achieved with group 2 herbicides and the group 14, diphenylether herbicides. These herbicide groups could be combined to improve the spectrum of weed control and manage herbicide-resistant weed species. Caution is warranted when applying these herbicides in mixture as herbicide actives from the imidazolinone and sulfonylurea families could result in reduced weed control when applied with the diphenylether herbicides (Nelson et al., 1998; Unland et al., 2000; Wesley, Shaw, 1992). The antagonism has been expressed primarily on annual dicot weeds but has also been documented on green foxtail [*Setaria viridis* (L.) P. Beauv] for the combination of imazamox with either acifluorfen or fomesafen (Unland et al., 2000). The antagonist of the latter would be the diphenylether herbicides as neither demonstrated appreciable activity on green foxtail when applied alone. In general, whole-plant research could not determine which herbicide was the basis for the interaction as both herbicides individually resulted in relatively similar levels of activity on the species (Nelson et al., 1998; Unland et al., 2000; Wesley, Shaw, 1992).

2.2.3 *Group 9 - Glyphosate*

Glyphosate has been regarded as one of the most effective foliar-active herbicides for control of annual and perennial weed species. Nonetheless, combining herbicides with glyphosate has progressively become more prevalent to provide soil residual activity, increase the speed of foliar activity, or target weeds that have become problematic due to the selection of glyphosate-tolerant or resistant weeds. Most of the herbicide antagonism documented with glyphosate has been in mixtures with group 2 herbicides. Applying group 2 herbicides, regardless of the chemical family, with glyphosate has produced antagonism mostly on dicot weeds, but also several grass species (Chachalis et al., 2001; Hydrick and Shaw, 1994; Kudsk, Mathiassen, 2004; Li et al., 2002; Norris et al., 2001; Rao, Reddy, 1999; Shaw, Arnold, 2002; Starke. Oliver, 1998). As with many antagonistic herbicide interactions, the reduction in weed control was corrected by increasing the dose of glyphosate in some instances (Shaw, Arnold, 2002).

The application of glyphosate with auxinic mimic (group 4) herbicides has been common for preplant burndown in no-till cropping systems and for industrial vegetation management. More recently, the commercialization of soybean traits that allow for in-crop applications of 2,4-D or dicamba with glyphosate has increased the frequency of these herbicide combinations. Mixtures of dicamba and glyphosate were antagonistic on kochia [*Kochia scoparia* (L.) Schrad.], susceptible and resistant to both glyphosate and dicamba (Ou et al., 2018). Similarly, the addition of the 2,4-D amine or ester formulations antagonized glyphosate activity on both GR and -susceptible populations of barnyardgrass (Li et al., 2020). This research highlights that if low-level resistance to glyphosate exists in a weed population, that the combination of glyphosate with an antagonistic herbicide could result in greater weed survival as a direct result of the herbicide interaction. Furthermore, the commercial practice of increasing the dose of glyphosate to control individual plants with suspected glyphosate resistance would be ineffective due to the antagonism from 2,4-D.

Combining dicamba with glyphosate reduced control of junglerice [*Echinochloa colona* (L.) Link] by 21%, and a similar reduction in glyphosate efficacy was observed when dicamba was replaced with 2,4-D (Perkins et al., 2021). Increasing the dose of dicamba intensified the grass antagonism of glyphosate instead of alleviating the interaction as has been reported for several interactions with other herbicide mode of action groups. Interestingly, related research demonstrated that applying glyphosate and either 2,4-D or dicamba simultaneously, yet in a separate boom system on the sprayer eliminated the antagonism on Italian ryegrass and broadleaf signalgrass (Merritt et al., 2020).

In the U.S. the legal requirements to apply dicamba in dicamba-resistant soybean with methods that minimize the risk for off-target movement of dicamba has further exacerbated challenges with herbicide performance in mixtures. Applying glyphosate and dicamba with spray tips producing ultra-coarse droplets and a drift reduction agent reduced control of junglerice by up to 56% compared

to glyphosate applied without dicamba or a drift reduction agent (Perkins et al., 2021). The requirement to use specific application methods for individual herbicides used in mixture may create a new type of interaction referred to as an "application" antagonism.

Use of group 14 herbicides (acifluorfen, flumiclorac, fomesafen, lactofen) in soybean production have historically been used for postemergence control of dicot weeds. In addition, fomesafen and sulfentrazone may have been used for preplant burndown of weeds before no-till planting. Combining these group 14 herbicides with glyphosate has resulted in antagonistic interactions across several monocot and dicot species (Chachalis et al., 2001; Norris et al., 2001; Starke, Oliver, 1998). Starke, Oliver (1998) concluded that glyphosate was being antagonized by these non-systemic herbicides with rapid foliar activity and limited glyphosate translocation.

2.2.4 *Group 10 - Glufosinate*

Glufosinate is a non-selective foliar herbicide used for preplant burndown prior to planting or in glufosinate-resistant crops. Glufosinate applied with group 2 (bensulfuron, halosulfuron), group 4 (triclopyr, quinclorac) and group 5 (metribuzin, propanil) herbicides has produced antagonism from control of several monocot and dicot weed species (Hydrick, Shaw, 1994; Lanclos et al., 2002). The specific antagonist in these mixtures was not clearly identified in the research, but the data suggest the tank-mix partners were antagonizing glufosinate, except in a few instances on rice flatsedge (*Cyperus iria* L.) when efficacy from glufosinate was less than the tank-mix partner applied alone.

2.2.5 *Group 22 herbicides*

The pyridinium herbicides that divert electrons from photosystem I (Group 22) have been used since the 1960s for non-selective weed management in terrestrial and aquatic sites. Combining paraquat or diquat with herbicides from group 1 (Buker et al., 2002), group 2 (Hydrick, Shaw, 1994), group 4 (Bovey et al., 1968; Bovey and Miller, 1968; Wehtje et al., 1992c), groups 5 or 6 (Buker et al., 2002; Hydrick, Shaw, 1994; Moore, Banks, 1991; Pritchard et al., 1980; Wehtje et al., 1992b), group 14 (Moore, Banks, 1991), and group 15 (Moore, Banks, 1991) created antagonistic mixtures for various monocot and dicot species. The antagonizing herbicide in these mixtures either varied by species or could not be determined from the data.

3. Mechanisms of herbicide interactions

Herbicide interaction can occur before the herbicide application due to physicochemical incompatibility or after herbicide application due to physiological interactions. In both cases, herbicide efficacy can be affected by the mixture.

3.1 Physicochemical incompatibility

Physicochemical incompatibility between active ingredients in a mixture can cause changes in the properties of the spray reducing the application effectiveness (Petter et al., 2012). These changes usually involve flocculation, phase separation, precipitation, and excessive foaming (Costa et al., 2020; Gazziero, 2015). The consequences of such changes include operational issues such as obstruction in the nozzles, screens, and filters, which can result in reduced herbicide efficacy because the concentrations of the active ingredient in spray droplets decreases (Petter et al., 2012).

Physicochemical incompatibility can also result in changes to the pH of the spray mixture, affecting the degradation, dissociation, absorption, and translocation of herbicides in the plant (Devkota, Johnson, 2019; Mueller, Steckel, 2019a). As an example, Glyphosate contains dissociable hydrogens, which can lead to interactions with charged components in the mixture and acidification of the medium due to the release of protons (Thelen et al., 1995). Mixtures of glyphosate with herbicides whose action is compromised under acidic pH should be avoided. For example, dicamba shows greater volatilization at pH<5.0 due to the formation of its acid form (Mueller, Steckel, 2019b), so this mixture should be avoided (Witten, 2019).

Physicochemical compatibility has been demonstrated some herbicides used in rice, such as cyhalofop-butyl, penoxsulam, pyrazosulfuron-ethyl, and imazethapyr, with no visible signs of incompatibility such as flocculation, sedimentation, phase separation, and foam formation in the tank (Rakes et al., 2017). However, adding imazapyr and imazapic to other herbicides used in rice cultivation reduced the pH of the mixture to values below 2.5, which can interfere with the effectiveness of the active ingredients (Rakes et al., 2017).

Likewise, mixtures of auxinic herbicides with glyphosate ammonium salt, as well as clethodim, did not exhibit visible physicochemical incompatibility, except for increased in foam formation in the tank, which was mainly observed in mixtures of 2,4-D choline and dicamba with two formulations of glyphosate (Avila Neto et al., 2021). While foam formation may not lead to less effective control, it can cause operational difficulties (Gazziero, 2015; Avila Neto et al., 2021). Another interaction involving glyphosate consists of mixtures with atrazine, one of the main herbicides used in maize. Sedimentation and lump formation occur immediately after these herbicides are mixed in the tank (Costa et al., 2020).

3.2 Physiological basis for herbicide synergism and antagonism – What do we know so far?

3.2.1 *Physiological basis for herbicide synergism*

The agrochemical industry has been particularly interested in capitalizing on potential synergisms between herbicides to increase the efficacy of their products. Scientists at Dupont Stine–Haskell Research Center studied the interaction between the photosystem II (PSII) herbicide atrazine and other inhibitors targeting various enzymes in the carotenoid biosynthesis pathways (Armel et al., 2007). The rationale behind this study was the known intricate relationships between carotenoids and plastoquinone biosynthesis and photosynthetic electron transport. Synergistic responses were observed when atrazine targeting the Q_B = binding site on photosystem II (#1 in Figure 2) was applied in mixtures with inhibitors of the MEP pathway (#2 in Figure 2) (the proherbicide clomazone, the active metabolite of clomazone, and fosmidomycin). Similarly, mixtures of atrazine and mesotrione, an p-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor (#4 in Figure 2) were synergistic. Other interactions had mixed results. For examples, combinations of atrazine with other inhibitors of carotenoid biosynthesis (i.e., phytoene desaturase (PDS), zeta-carotene synthase (ZDS), and lycopene cyclase (LC) inhibitor (#3 in Figure 2) were synergistic on some species but antagonistic on other species (Armel et al., 2007).

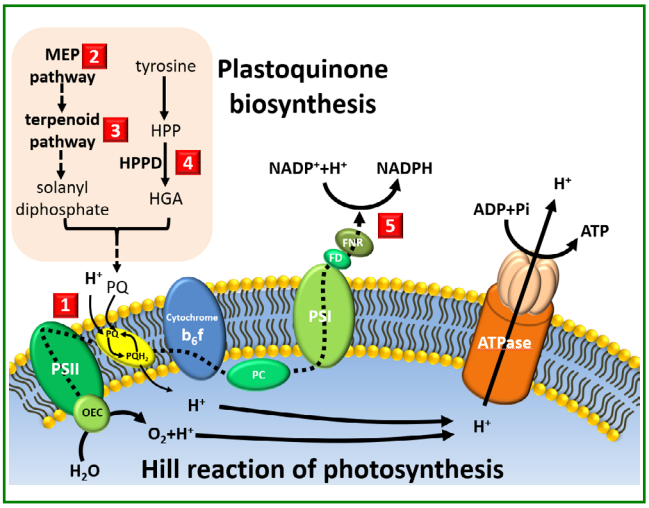

Figure 2 - Illustration of the relationship between photosystem II (PSII) and photosystem I (PSI) of the Hill reaction in the thylakoid membrane, as well as a summary of the pathways leading to plastoquinone biosynthesis. Abbreviations: OEC, oxygen evolution complex; HPP, hydroxyphenyl pyruvate; HGA, homogentisate; HPPD, hydroxyphenylpyruvate dioxygenase; PQ, plastoquinone; PQH_2 = plastoquinol (reduced plastoquinone); PC, plastocyanin; FD, ferredoxin; FNR, ferredoxin-NADP reductase. Red squares with numbers indicate target sites with known synergism. 1= Q_B binding site of group 5/6 inhibitors; 2= target of inhibitors in the MEP pathway (groups 13); 3= target of inhibitors in the terpenoid/carotenoid pathway (groups 12, 34); 4= target site of HPPD inhibitors (group 27); 5= target site

A recent study from the University of Western Australia described a systematic approach to investigate synergistic herbicide interactions and differentiate these from the more common additive or antagonistic relationships (Sukhoverkov, Mylne, 2021). Their study using 24 commercial herbicides were used to create a matrix of all 276 unique combinations to search for new synergies. Their main conclusion was that synergistic interactions are relatively rare, and the interaction depends on the plant species and herbicide dose.

The most well-known synergy is the one involving inhibitors of photosystem II (PSII; group 5/6) and hydroxyphenylpyruvate dioxygenase (HPPD; group 27) (Abendroth et al., 2006; Hugie et al., 2008). This interaction is so positive that mixtures can be used to overcome herbicide resistance to these individual modes of action. The physiological basis for this synergistic interaction is multifold. One of the key features of photosynthesis is the linear electron transport between PSII and PSI that is required for NADPH synthesis. Group 5/6 herbicides bind to the Q_B = binding site on PSII (#1 in Figure 2) and compete for the binding of plastoquinone, a molecule involved in electron transport between PSII and cytochrome b6f. Group 27 herbicides inhibit plastoquinone biosynthesis (#4 in Figure 2). These herbicides caused bleaching of plants because plastoquinone is required for phytoene desaturase activity (a key enzyme in carotenoid biosynthesis), and carotenoids are required for stabilizing the photosystem. Furthermore, plastoquinone is involved in photosynthetic electron transport. Consequently, mixtures of group 5/6 herbicides with group 27 are synergistic because the inhibition of plastoquinone synthesis both decreases levels of carotenoids necessary for stabilizing the photosystem and reduces the pool of free plastoquinone, making the binding of group 5/6 herbicides to PSII more potent. There is also some evidence that atrazine increased mesotrione absorption (Chahal et al., 2019).

Photosystem II (PSII; group 5/6) inhibitors may also interact with photosystem I (PS I; group 22) inhibitors (#5 in Figure 2). Under most circumstances, these interactions are antagonistic (see antagonism section). However, under certain conditions, the reduction in electron transport caused by a PSII inhibitor may reduce the contact activity of a PSI inhibitor such as paraquat, enhancing its translocation and resulting in overall better weed control over the long-term, as was demonstrated with tank mixtures of paraquat and simazine on quackgrass (Putnam, Ries, 1967).

A recent investigation on the mode of action of glufosinate (group 10) revealed an interesting interaction between inhibition of glutamine synthetase and protoporphyrinogen oxidase (PPO inhibitors, group 14) (Takano et al., 2020b). Glutamine synthetase (GS), a key enzyme for amino acid metabolism and photorespiration, catalyzes the incorporation of ammonia in glutamate to form glutamine. Inhibition of GS deregulates ammonia metabolism and shunts a large amount of the

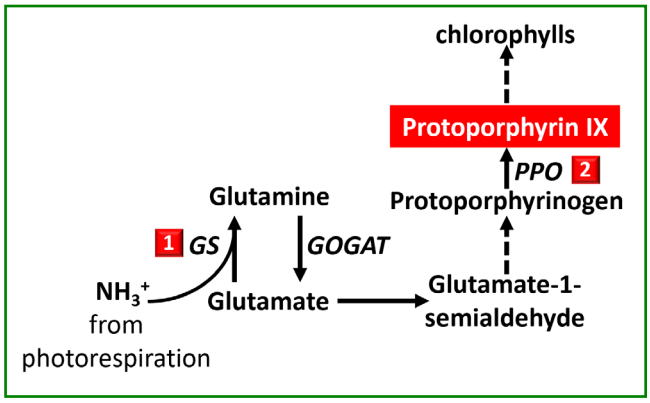

Figure 3 - Biochemical relationship between glutamine biosynthesis and porphyrin biosynthesis. Inhibition of GS by glufosinate shunts large amount of glutamate toward chlorophyll synthesis, causing higher herbicidal injury associated with herbicides inhibiting PPO. Abbreviations: GS, glutamine synthetase; GOGAT, glutamine oxoglutarate aminotransferase; PPO, protoporphyrinogen oxidase. Dotted lines indicate more than one enzymatic step

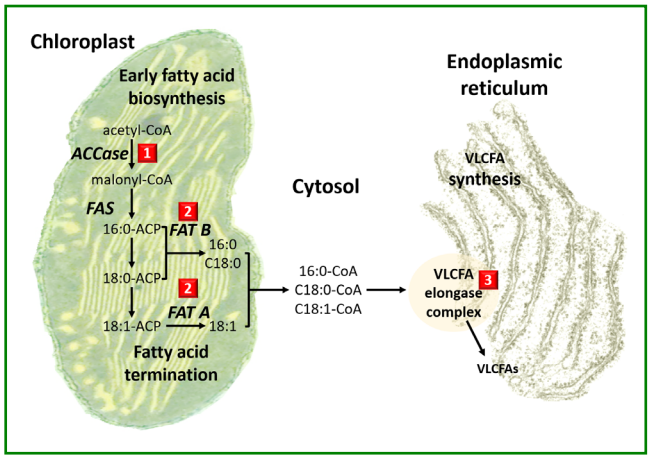

Figure 4 - Illustration of the relationship between different steps of fatty acid biosynthesis and elongation. Abbreviations: ACCase, acetyl-CoA carboxylase; FAS, fatty acid synthase; FAT, fatty acyl thioesterase; VLCFA, very-long chain fatty acid. Red squares with numbers indicate target sites with known synergism. 1= target site of ACCase inhibitors (group 1); 2= target site of FAT inhibitors (group 30); 3= target site of VLCFA elongases inhibitors (group 15) (Figure adapted from Campe et al., 2018)

free glutamate toward the biosynthesis of chlorophyll (Figure 3). This rerouting of glutamate occurs because each chlorophyll molecule is composed of 8 glutamate units required for the biosynthesis of the porphyrin backbone. Consequently, glufosinate enhanced the activity of low doses of protoporphyrinogen oxidase (PPO) inhibitors (group 14) by causing a dramatic increase in the accumulation of the highly photodynamic protoporphyrin intermediate. The synergism between the two herbicides was also confirmed by isobole analysis and field trials. The herbicide combination provided high levels of efficacy when applied at low temperature and low humidity (Takano et al., 2020b).

Fatty acid biosynthesis is another biochemical pathway with multiple herbicide target sites that may be suitable for synergistic interactions. Acetyl-CoA carboxylase inhibitors (ACCase, group 1) inhibits the first and rate-limiting step in fatty acid biosynthesis, converting acetyl-CoA to malonyl-CoA (Figure 4). While there are no commercial inhibitors of the next several steps catalyzed by fatty acid synthase, it was recently discovered that cinmethylin inhibits fatty acyl thioesterases (FAT, group 30) involved in termination of fatty acid biosynthesis in the chloroplast (Figure 4). Finally, several classes of herbicides inhibit very long-chain fatty acid elongases (VLCFAE, group 15) in the endoplasmic reticulum. While there is no case of synergy between ACCase and FAT inhibitors to date, it has been reported that ACCase and VLCFAE inhibitors can synergize one another. For example, tank mixtures of the VLCFAE inhibitor dimethenamid with either of the ACCase inhibitors fluazifop-P and sethoxydim synergized broadleaf signalgrass (Scott et al., 1998a). Mechanistically, inhibition of ACCase will reduce the carbon flow through fatty acid biosynthesis, which will lower the free pool of fatty acids entering the endoplasmic reticulum for elongation by the VLCFA elongase complex. Because VLCFAE inhibiting herbicides bind covalently (and irreversibly) to their targets, it is likely that their effect may be more rapid and with greater consequences when used in a tank mix with ACCase inhibitors (Schmalfu et al., 2000).

A synergistic effect was observed between the glyphosate (group 9) and the PPO inhibitor saflufenacil (group 14) in the management of GR hairy fleabane (Dalazen et al., 2015). The combination of these chemistries resulted in faster and greater oxidative stress and lipidic peroxidation compared with the effects of either herbicide alone (Piasecki et al., 2020). However, there are also many antagonism examples between glyphosate and other PPO herbicides.

Other cases of synergism are associated with enhanced translocation of one of the active ingredients. For example, a biochemical interaction cannot explain the synergistic interaction between the auxin herbicide 2,4-D amine with the PSII-inhibiting herbicide metribuzin on winter wild oat (*Avena sterilis* L.) between the two mechanisms of action nor by changes in foliar absorption of metribuzin. However, the mixture resulted in enhanced metribuzin translocation to the roots and new leaves. Since new leaves express high levels of the psbA gene to prepare this nascent tissue for photosynthesis, the synergism is most likely due to the increased metribuzin translocation to new leaves, even though enhanced metabolism may offset some of the effect (Han et al., 2020).

It is often the case that one herbicide may improve the activity of another by altering the rate of herbicide

metabolism. For example, a synergistic interaction between thiobencarb (VLCFAE, group 15) and bispyribac-sodium (ALS, group 2) were assessed on late watergrass [*Echinochloa phyllopogon* (Stapf) Koss.] in California rice. This positive interaction may be associated with the effect of the thiocarbamate enhancing the activity of enzymes in Phase I herbicide metabolism (Fischer et al., 2004).

Finally, there are many reports characterizing synergistic interactions between different herbicides, but these papers do not address the possible underlying physiological and/or biochemical bases for these observations. For example, there is a report of synergy between glyphosate (group 9) and the synthetic auxin fluroxypyr (group 4) for mallow (*Malva parviflora* L.) control (Chorbadjian, Kogan, 2002). Another example shows the joint action of glyphosate (group 9) and the metsulfuron-methyl (group 2). While the mechanism behind this interaction was not elucidated, data pointed to the potential role of the formulations in the spray solution (Kudsk, Mathiassen, 2004).

3.2.2 *Physiological basis for herbicide antagonism*

There are several causes of herbicide antagonism, but reduced uptake and/or translocation is by far the most common. This results from the application of one chemical that reduces the uptake and/or translocation of an herbicide (Table 2). The negative interaction between glyphosate and dicamba on glyphosate-susceptible and -resistant *Bassia scoparia* L. was caused by decreased translocation of the two herbicides resulting in reduced efficacy with the herbicide combination compared to the individual herbicide treatments (Ou et al., 2018). Similarly, dicamba, atrazine + dicamba, or atrazine + bentazon decreased ^{14}C-sethoxydim absorption by 9 to 63% across giant foxtail, large crabgrass, shattercane, and corn (Young et al., 1996). The antagonism

Table 2 - Physiological basis for antagonism between more than one herbicide in tank-mixtures across different weed species.

Physiological basis	Antagonizing herbicide	Antagonized herbicide	Species	Reference
Reduced uptake and/ or translocation rates	Glyphosate Dicamba	Dicamba Glyphosate	*Bassia scoparia* (L.) A.J. . Scott	Ou et al., 2018
	Atrazine	Nicosulfuron	*Setaria viridis* (L.) P.Beauv.	Schuster et al., 2007
	Glufosinate	Glyphosate	*Setaria faberi* Herrm.	Besançon et al., 2018
	Dicamba, Dicamba + atrazine Atrazine + Bentazon	Sethoxydim	*Setaria faberi* Herrm. *Digitaria sanguinalis* (L.) Scop. *Sorghum bicolor* (L.) Moench ssp. *verticilliflorum* (Steud.) de Wet ex Wiersema & J. Dahlb. *Zea mays* (L.)	Young et al., 1996
	Pyrithiobac-sodium	Fluazifop	*Digitaria sanguinalis* (L.) Scop.	Ferreira et al., 1995
	Tribenuron	Diclofop	*Avena fatua* (L.)	Baerg et al., 1996
	Dicamba 2,4-D	Glyphosate	*Sorghum halepense* (L.) Pers.	Flint and Barrett, 1989b
	Bentazon	Haloxyfop	*Setaria pumila* (Poir.) Roem. & Schult.	Gerwick, 1988
	2,4-D amine	Glyphosate	*Hordeum vulgare* (L.)	O'Donovan and O'Sullivan, 1982
	Propanil	Pyribenzoxim	*Echinochloa colona* (L.) Link	Koo et al., 2000
	Bromoxynil	Quizalofop	*Setaria pumila* (Poir.) Roem. & Schult.	Culpepper et al., 1999
	Naptalam	Paraquat	*Arachis hypogaea* (L.)	Wehtje et al., 1991
Reduced metabolism	Propanil	Cyhalofop-butyl	*Echinochloa crus-galli* (L.) P. Beauv.	Ottis et al., 2005
Increased metabolism	2,4-D	Diclofop	*Avena fatua* (L.)	Busi et al., 2017
Effect on photosynthesis	Paraquat	Atrazine	*Arachis hypogaea* (L.)	Wehtje et al., 1992b
	Trifloxysulfuron Imazapic	Clethodim	*Eleusine indica* (L.) Gaertn.	Burke and Wilcut, 2003
Chemical interaction	Na-Bentazon	Sethoxydim	*Elymus repens* (L.) Gould	Wanamarta et al., 1989

of fluazifop activity by pyrithiobac-sodium was caused by decreased translocation of fluazifop out of the treated leaf in large crabgrass (Ferreira et al., 1995). Glyphosate uptake into johnsongrass leaves and subsequent translocation to the roots was reduced by the presence of 2,4-D or dicamba (Flint, Barrett, 1989b). Tribenuron decreased basipetal translocation of diclofop from the treated zone by approximately 20% in wild oat (Baerg et al., 1996). Sequential application of naptalam before an application of paraquat, as well as absorption studies utilizing ^{14}C-paraquat, indicated that the antagonism was due largely to reduced paraquat absorption (Wehtje et al., 1991). Finally, antagonism of quizalofop activity by bromoxynil is primarily due to decreased absorption of quizalofop, whereas effects on translocation and metabolism were minor (Culpepper et al., 1999).

One common way that an herbicide can affect the translocation of another is by rapid desiccation of the leaf surface. Reduction in efficacy when nicosulfuron was applied in combination with mesotrione + atrazine was due to decreased absorption and translocation of nicosulfuron in green foxtail (Schuster et al., 2007). The reduction in the absorption of sulfonylurea herbicides when mixed with mesotrione + atrazine was due to the destruction of epidermal cells at the point of contact. Atrazine is poorly translocated into the plant and acts primarily by indirectly enhancing lipid peroxidation and causing cell membrane disruption. This disruption results in rapid cell death at the point of contact, and sulfonylurea herbicides can become tightly adsorbed to dead tissue. Translocation of sulfonylurea herbicides in plants is an active process, but lower concentrations of sulfonylurea herbicides in plant tissue result in decreased translocation from the point of contact to meristematic regions (Schuster et al., 2007). Another example of reduced uptake and translocation by the rapid action of one of the herbicides occurs with glufosinate. Like atrazine, glufosinate is a contact herbicide that leads to rapid cell death by reactive oxygen species (Takano et al., 2020c), which can limit the uptake and translocation rates of any herbicide including glyphosate. Reduced glyphosate translocation rates were responsible for the antagonism observed between glyphosate and glufosinate in giant foxtail (Besançon et al., 2018).

Similar observations were made in a study evaluating potential synergism between saflufenacil and glyphosate on buckwheat (*Fagropyrum esculentum* Moench.). Glyphosate reduced the translocation of saflufenacil, and the contact activity of saflufenacil reduced the activity of glyphosate, possibly by reducing its translocation. Thus, the antagonism of saflufenacil and glyphosate involves two mechanisms that altered absorption and translocation (Ashigh, Hall, 2010).

Antagonism is often related to the physicochemical properties of the spray solution. For instance, less glyphosate was retained on the leaf surface of barley when glyphosate was applied in combination with the complete formulation or solvent system of 2,4-D amine, as compared to glyphosate alone or glyphosate in combination with the technical component of the 2,4-D amine formulation (O'Donovan, O'Sullivan, 1982). Tank-mixing sethoxydim with the sodium salt of bentazon reduced the activity of sethoxydim in controlling a wide variety of weedy grass species in the field. The interaction between sethoxydim and the sodium salt of bentazon occurred when the Na+ ions from the sodium salt of bentazon presumably exchanged with the H^+ of hydroxyl group of sethoxydim and formed the sodium salt of sethoxydim. The absorption of the sodium salt of sethoxydim was inhibited because it is more polar than sethoxydim. Therefore, tank-mixing the sodium salt of bentazon with sethoxydim reduced the absorption and activity of sethoxydim (Wanamarta et al., 1989).

Another interesting mechanism of antagonism interaction between herbicides occur when a compound interferes with metabolism rate of another. Herbicide antagonism was also observed with cyhalofop-butyl and propanil controlling barnyardgrass. Propanil decreased apoplastic esterase enzyme activity, reducing the metabolism rate of cyhalofop-butyl into cyhalofop-acid (Ottis et al., 2005). In contrast, the 2,4-D pre-treatment induced enhanced rates of diclofop-methyl metabolism into diclofop-acid (Busi et al., 2017). This is like a metabolic resistance mechanism determined for field evolved ACCase *herbicide-resistant* rigid ryegrass populations. 2,4-D also induced cross protection against the ALS herbicide chlorsulfuron (Group 2), which can be reversed by the known P450 inhibitor malathion, further implicating P450 involvement (Busi et al., 2017). Therefore, herbicide antagonism between two or more chemicals can result from either increased or decreased metabolism rates of each.

Multiple mechanisms can occur in the same herbicide combination. For example, the efficacy of ACCase-inhibiting herbicides was reduced by 2,4-D or MCPA in wild oat and other grass species. Various mechanisms have been proposed including recovery from membrane depolarization (Shimabukuro et al., 1987), reduction in herbicide uptake or translocation (Todd, Stobbe, 1980) and altered herbicide metabolism including reduced diclofop-methyl de-esterification (Kafiz et al., 1989). In addition, the ACCase-inhibiting aryloxyphenoxypropionates can interfere directly with the processes controlling proton-efflux in the *Avena sativa* L. coleoptile and act as competitive inhibitors of synthetic auxin-receptor binding suggesting that the basis of antagonism may reside at the receptor level (Barnwell, Cobb, 1993).

Finally, herbicides can interfere with other physiological processes (i.e. photosynthesis) and therefore compromise the mode of action of another herbicide in the tank mix. For instance, antagonism of clethodim by trifloxysulfuron-sodium was observed in goosegrass and physiological investigation suggested

that the ALS inhibitor may prevent the herbicidal activity of the ACCase herbicide clethodim, causing the antagonism effect (Burke, Wilcut, 2003). In the same study, clethodim absorption, translocation, and metabolism were not affected by the presence of trifloxysulfuron-sodium. Photosynthetic rates of goosegrass, however, were reduced by trifloxysulfuron-sodium treatment. By the time plants had recovered to normal growth and photosynthesis, no active clethodim remained in the plant. Moreover, tank-mixing paraquat with PSII inhibitors often leads to antagonism or additive effect in plants (Wehtje et al., 1992b). Paraquat needs to accept light-generated electrons to become phytotoxic, when mixed with PSII inhibitor, it reduces the electrons flow and reduce the electrons that reaches paraquat.

4. Methods for evaluating physiological interactions between herbicides

4.1 Concepts of reference models

The most common reference models are the Additive Dose Model (ADM) and the Multiplicative Survival Model (MSM). Morse (1978) described and compared those two models. Outside the discipline of weed science, ADM is denoted Concentration Addition (CA), and MSM is denoted Independent Action (IA). Within weed science, the MSM is denoted Colby's Method.

4.1.1 *Additive Dose Model or Concentration Addition*

ADM answers the question raised by many end-users. "If I use a recommended rate of 2.5 kg of herbicide A and 0.5 kg of herbicide B per unit area, applied separately to yield a 90% weed control, which mixture ratios of the two could I use without gaining or losing effects?" In the quest for documenting antagonistic or synergistic effects, ADM unambiguously defines any ratio of an herbicide in a mixture from zero to 100% of a herbicide. Furthermore, when herbicide ratios approach either 0 or 100%, the response curves revert to either of the two herbicides administered separately.

The ADM assumes that two herbicides with the same mode/site of action do not interfere with each other's action at the enzymatic level, all other things being equal. A straight-line isobole is derived from the two herbicides administered separately. It represents all combinations of ratios for a particular effect level, e.g., ED_{10}, ED_{50}, and ED_{90}, to denote NOEL (No Observable Effect Level), general toxicity, and appropriate weed control level. The isobole defines all possible combinations of the two herbicides that yield the same response level. The isobole is essentially a contour plot at a specific response level that illustrates all combinations of two herbicides giving the same response in a plant (Figure 5). We use a sigmoid dose-response curve, e.g., a log-logistic dose-response model, to describe

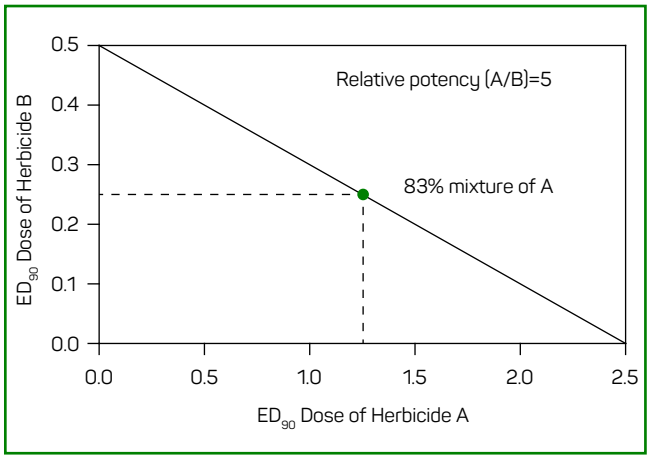

Figure 5 - ED_{90} isobole for herbicide A and B with a mixture halfway between the ED_{90} for herbicide A (2.5) and herbicide B (0.5). The actual mixture ratio is 83% of A and thus 17% of herbicide B. Because of the placement on the isobole, we often call it a virtual 50% mixture. It illustrates if you do not know the relative potency between the herbicides, you do not know where you are on the isobole. Mixtures being situated to the right of the Additive Dose Model (ADM) isobole are antagonistic, and to the left of the ADM, synergistic

the dose-response curves of herbicides and mixtures (Ritz, 2010; Ritz et al., 2015).

In the example above, at the ED_{90}, the mixture ratio coincides with the isobole halfway between the A and B herbicides (A=1.25 and B=0.25 kg per unit area). Accordingly, the term additive in ADM is not based on the sheer addition of the doses but involves the relative potency between the doses obtaining the same effect (eq. 1). A ratio of the mixtures halfway between herbicide A and B in Figure 5 would be 1.25/(1.25+0.25) = 0.83 or 83% of A. In other words, herbicide B is five-fold as potent as is herbicide A. It is defined for ED_{90}:

$$r_{ED90} = \frac{Z_A}{Z_B} \qquad \text{(eq.1)}$$

where Z_A and Z_B are the doses of the two herbicides applied separately at ED_{90} for the two herbicides.

The relative potency, r_{ED90}, between two herbicides can also be considered the biological exchange rate and denotes the relative displacement of the two response curves for the herbicides applied separately at ED_{90}. The ADM isoboles could be defined by the relationships below (Hewlett, Plackett, 1979):

$$\left(\frac{Z_A}{Z_A}\right)^{\lambda A} + \left(\frac{Z_B}{Z_B}\right)^{\lambda B} = 1 \qquad \text{(eq. 2)}$$

where Z_A and Z_B were defined earlier; z_A and z_B are the doses of A and B in a mixture. The exponents, λ_A, and λ_B, could be the same or different, symmetric or asymmetric, respectively. If the exponents in eq. 2 are similar, viz.

symmetric isobole, if λ >1.00, we have synergism, and if λ < 1 we have antagonism (Figure 5).

With ADM, the herbicides can be considered a dilution of each other, i.e., they have the same action in the organism, or to be more specific, they share the same target site, and the herbicides do not compete at the binding site. It can mathematically be expressed by:

$$Z_A = r \cdot Z_B = z_{mix}\,(p + r \cdot (1 - p)) \qquad \text{(eq. 3)}$$

where r is the relative potency and a predefined ED_x level, p is the proportion of herbicide A in the mixtures, and the z_{mix} is the sum of the herbicides in a mixture. A z_{mix} dose can be expressed unambiguously by a biologically equivalent dose of either herbicide applied alone (eq. 3). It applies to any dose-response relationship as long as the response curves are either monotonically decreasing or increasing.

The ADM (eq. 3) can be incorporated into a dose-response model with similar dose-response curves of the herbicides when mixtures are in a fixed ratio identified by p. If λ_A and λ_B are different, the isoboles are not symmetric (Hewlett, Plackett, 1979). Another way of describing asymmetric isoboles is to use the so-called Vølunds model (Streibig et al., 1998).

If the dose-response curves for herbicides administered separately are not similar, the isoboles at various ED_x levels change because r is ED_x dependent (Ritz et al., 2006). The isoboles are straight lines at any response level, but their slope changes with the ED_x level. Another problem of working with non-similar dose-response curves for the herbicides administered separately is that the mixture curves with herbicides in fixed ratios do not follow the same mathematical relationships as the curved for the herbicides administered separately.

In herbicide research and development, the mode of action, i.e., biomass response, is affected by a multitude of the sites of action. However, when focusing on one mode of action, e.g., biomass response, we reduce all herbicides to having the same mode of action. The definitions of site and mode of action are equivocal; they are dose-dependent. As Fedtke (1982) pointed out, an herbicide might have primary, secondary, and even tertiary sites of action depending on the size of the doses. Thus dose-response curves from virtually no effect at low doses to complete kill at high doses might be a mix of those sites of action. Whether the premises are on the enzymatic level, ADM is a straightforward method that gives the researcher and herbicide end-user operational results.

When two herbicides have the same site of action, their dose-response curves should be similar apart from their relative horizontal displacement. It means that two dose-response curves have the same regression parameters except for the relative displacement of the curves along the dose axis. Consequently, one can say that two similar dose-response curves are a necessary but not sufficient prerequisite for documenting a similar site of action (Streibig, 1984).

From a statistical point of view, the ADM can equally well be used for the continuous and binomial responses. Still, we must find proper statistical tests to substantiate departure from additivity (Ritz et al., 2021).

4.1.2 *Multiplicative Survival Model or Independent Action*

If two herbicides produce their effects in entirely differently ways, although the measured response is the same for both herbicides, one might expect a contrasting model when they are acting together. It is MSM (Colby, 1967; Finney, 1971; Hewlett, Plackett, 1979; Morse, 1978). The reference for MSM is also the dose-response curves for two herbicides administered separately. Still, MSM requires that the responses be expressed as a proportion of a hypothetical maximum value. MSM was developed for binary responses (dead or alive, affected not affected) and not continuous responses. It fits into the mortality of insects in response to dose and the infected plants in phytopathology.

In weed science, the response is often continuous such as biomass, e.g., length, physiological, and biochemical responses. Therefore, the use of MSM can only be considered an approximation in those situations. As Morse (1978) points out, the untreated control on a continuous scale is subject to error, and therefore relative response at small doses could exceed that of the untreated control. The relative potency, r in eqs 1 and 3) does not play a role for MSM. MSM assumes the two herbicides act independently without interfering with each other's actions. Berenbaum (1981) illustrated it like this: "one throws bushels of pebbles or nails at a collection of eggs." The pebbles and nails represent two different herbicides. None of the nails or pebbles cooperate in cracking the eggs, and the cumulative damage is merely a matter of combining probabilities. Suppose an herbicide mixture contains a dose of x of herbicide A and z of herbicide B when applied alone. P_A and P_B would be the proportions of the plants responding to the herbicides A and B (eq.5). The mixture response of the two herbicide doses (x, z) would be P:

$$(1 - P) = (1 - P_A) \cdot (1 - P_B) \qquad \text{(eq. 5)}$$

MSM is based on the multiplication rules for probabilities of independent events. If not dealing with binomial responses (alive/dead, affected/ not affected), the scaling of responses relative to the response at zero doses reduces to the product of the dose-response models (Ritz et al., 2021) (eq. 6):

$$f_{Ind}\,(x,z) = f_A\,(x) \cdot f_B\,(z) \qquad \text{(eq. 6)}$$

The mixture dose-response model, f_{ind}, will not have the same form as the dose-response curve of either f_A or f_B for the individual herbicides. It is almost the same as for ADM; if the log-logistic dose-response models are not similar, the dose-response for any one mixture ratio would not be exactly a log-logistic curve. From a practical point of view, this might not be a problem, but it is worth

considering if the dose-response curves for the individual herbicides differ dramatically. An illustration of the results in eq. 6 could be a contour plot that gives various ED_x - response levels.

4.2 Experimental design and analysis

We must plan experiments without knowing the proper dose range of the herbicides applied alone or in mixtures. However, the ray design in (Figure 6), allows one to control how well the dose-response curves fit and the distribution of mixtures ratios covering the isobole (Figure 6). The largest deviation from the isoboles, whatever the choice of the reference model, is in the middle of the isobole. Even though the relative potency does not play a role in the MSM, it would be wise to know the relative potency to distribute the mixture ratios over the whole response area. If the ratios of mixtures are displaced to either of the dose axes in Figure 5, the chances of detecting departures from the isobole are difficult. Abdelbasit and Plackett (1982) discussed distributions of ratios, so the ratios cover the isobole evenly from one axis to the other by using either log (ED_{50}) or ED_{50} of the individual herbicides.

MSM has gained popularity because it is easy to get results from a factorial analysis of variance with two herbicides at various doses and combinations. Amazingly it is still used in patent applications. However, the ANOVA does not model dose-response curves; it only compares

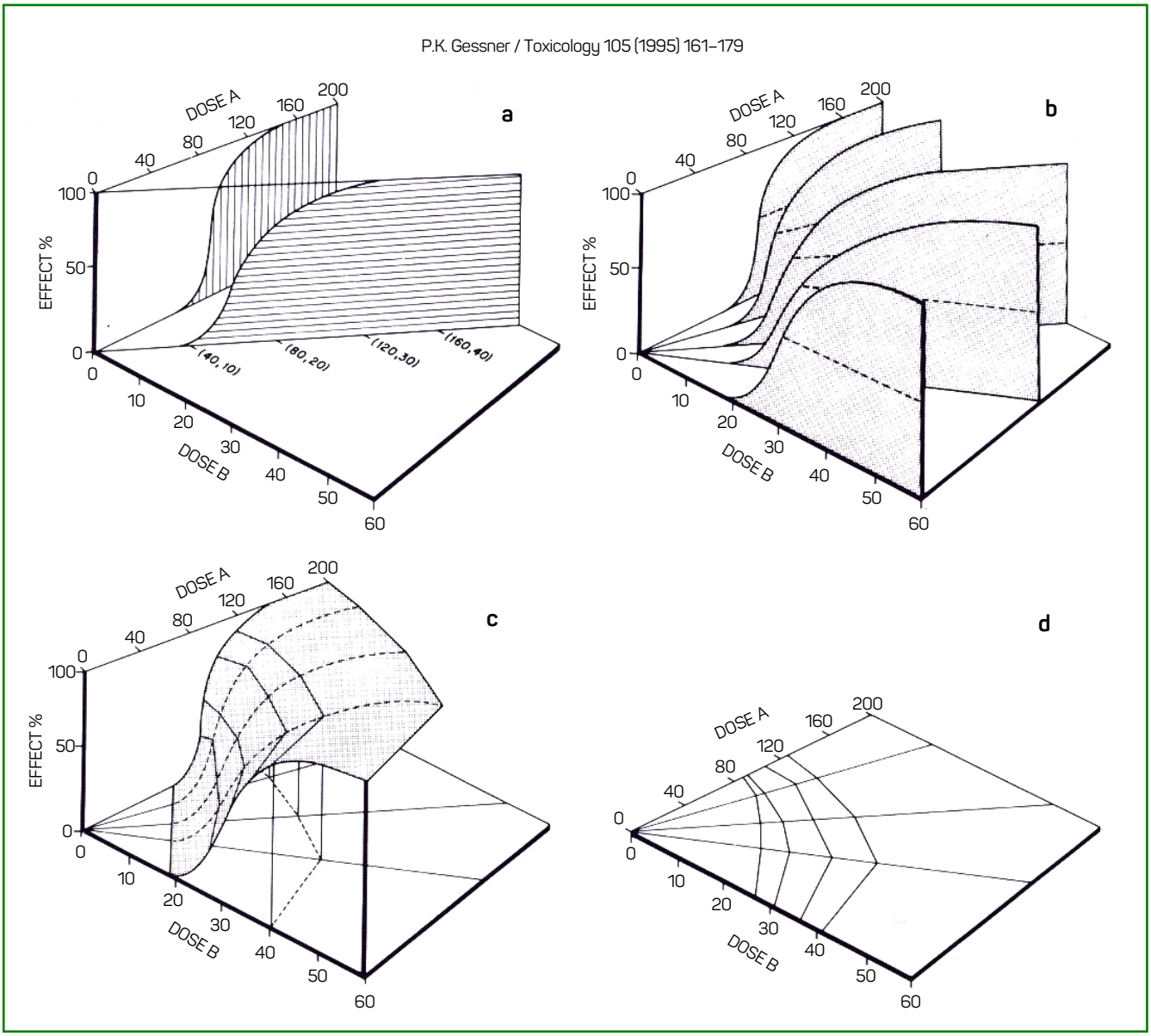

Figure 6 - An illustration of a ray design and the derived isoboles. The dose-response curves of two herbicides are shown in a). For fixed mixing ratio between A and B, b). The contour plots, c). The projection of the isoboles at various ED_x response levels in c). From Gessner (1995). Used under permission (License number 5430840650404)

differences in effects at particular dose combinations. The results are point estimates.

The question arises if the differences between ADM and MSM can be substantiated in practice. Consequently, it is crucial to run the experiments twice to get an idea of the variability (Cedergreen, Streibig, 2005). The fact is that a bioassay with mixtures gives a snapshot of the action of mixtures, all other things being equal. However, independently running the same experiment under slightly different conditions gives a good idea of the inherent variability. Unfortunately, when reporting synergism or antagonism, only a few papers satisfy this relatively simple prerequisite (Cedergreen et al., 2007a).

4.3 Multi-mixtures

Multiple mixtures are analyzed along the same lines as the binary mixtures, but the results are difficult to illustrate and interpret. One way to get around this problem is for ADM to use Toxic Units (TU). It can be calculated on any ED_x for any mixture ratio and any number of herbicides in a mixture. It has been used somewhat in ecotoxicology (Cedergreen et al., 2012), but not yet used in herbicide science to optimize mixtures. It is based upon the ADM equation in eq. 2.

$$TU = \left(\frac{Z_A}{Z_A}\right) + \left(\frac{Z_B}{Z_B}\right) + \ldots \left(\frac{Z_N}{Z_N}\right) \quad \text{(eq. 7)}$$

where TU is the Toxic Unit, and z_A to z_N is the sum of herbicides in the mixture at a defined ED_x response level. Similarly, Z_A to Z_N is the doses of the herbicides administered separately. The number of herbicides in a mixture follows the ADM if the TU is not different from 1.

5. Herbicide mixtures and weed resistance

Mixtures of herbicides with different mechanisms of action are one of the most important strategies for delaying the evolution of weed resistance (Wrubel, Gressel, 1994). In comparison with herbicide rotation, herbicide mixtures are more efficient for delaying the herbicide resistance (Diggle et al., 2003). One of the main limitations for using herbicide mixtures for controlling herbicide resistance is the occurrence of antagonism on weeds and synergism on crops, which decrease the control efficiency and selectivity, respectively. The synergistic effect of mixtures on crops is related to the undesirable herbicide effect of the herbicides on crops, which is few reported in the literature. However, the occurrence of an antagonistic effect of herbicide mixtures on weed control was found in several studies (Matzenbacher et al., 2015; Zhang et al., 1995). This occurrence raises a question about the effect of certain herbicide mixtures favoring the evolution of resistance. If the herbicide effect is decreases, it may favor the survival of individual exposed to the herbicide. Recently, a study evaluated the effect of low doses of the mixture of fenoxaprop-p-ethyl and imazethapyr applied generation after generation on susceptible and resistant barnyardgrass populations. The results indicated that control with the herbicide mixture of second-generation progenies of a susceptible biotype and mainly in a resistant biotype was lower in comparison with the effect of each herbicide alone (Rigon, 2019). Therefore, the improvement of herbicide resistance management requires knowledge of not only the effect of mixtures for controlling herbicide resistance biotypes but also about the effect of certain mixtures as a factor that may increase the evolution of herbicide resistance.

Another strategy of mixtures related with herbicide resistance is the use of compounds directly associated with the mechanism of resistance. The organophosphate compounds piperophos and anilofos in mixtures with propanil improved the control of propanil-resistant junglerice (Valverde, 2007). A commercial formulation of propanil plus piperophos was registered in early 1990s and extensively used in several countries of South and Central America until the availability of ALS-inhibitors selective for rice (Valverde, 2007). A similar approach was developed with PPO-inhibitors, but the interest of industry for patent and commercial registration were restricted due to the actual toxicological restrictions of organophosphate compounds (A. Merotto Jr., personal information). This is an example of the effect of broad prohibitions of using certain products due to toxicological restriction may result in increasing overall pesticide use. The consideration of the problems related with herbicide resistance and the absence of discovering new herbicide mechanisms of action should be considered in a case-by-case analysis of the synergistic compounds that result in increasing the diversity of herbicides for controlling the most troublesome weed species. The common target for synergistic mixtures to overcome resistance is the cytochrome P450 monooxygenases, and, unfortunately, all available inhibitors of these group of enzymes are old fashion insecticides considered as highly toxic.

A large avenue exists to identify other CytP450 and herbicide detoxification inhibitors and other inhibitors of transmembrane herbicide movement recently associated with resistance recently reviewed by Torra et al. (2021). In medicine, where no new classes of antibiotics have been developed in the last 30 years (Liu et al., 2021) synergistic mixtures are largely used to combat several diseases. The mixture of amoxicillin and clavulanate has been used for more than 40 years, where the clavulanate role is to inhibit the amoxicillin degradation (Veeraraghavan et al., 2021). The overuse of this combination has also selected for resistance and a recent third-generation mixture of these compounds with cephalosporins has been developed (Veeraraghavan et al., 2021). The main strategies to confront multi-drug resistance related to mixtures of non-antibiotic compounds are based on resistance inhibitors (clavulanate), membrane disrupters (pentamidine), signaling inhibitors (Fe-PcTs), and immune enhancer

(streptazolin) (Liu et al., 2021). These are examples about how the use of non-target compounds is important for combating the resistance evolution. Some of the new potential herbicides with new mechanisms of action discovered are not effective on certain weed populations due to the occurrence of metabolism-based herbicide resistance, resulting in rejection for developing these compounds despite the novelty regarding the mechanism of action. Thus, a large effort may occur not only for discovering new efficient herbicides mechanism of action but also, or more important, for discovering non-herbicide agents that act on the mechanism of metabolism-based herbicide resistance for using in mixtures. Therefore, the mixture theories discussed in this article will be the core of the future herbicide science.

6. Conclusions

The most used reference models for herbicide interaction are ADM and MSM, and the choice of one or the other affects the interaction interpretation of the mixture.

The prediction of herbicide interactions is complex and is not always possible, due to the interference of different factors in the response obtained.

Antagonistic interactions can increase the evolution of weed resistance by favoring the survival of individuals exposed to an herbicide.

Physicochemical incompatibility in the spray tank usually causes herbicide antagonism, whereas both synergism and antagonism can result from increased or decreased uptake/translocation and from physiological changes in the plant.

7. Author's contributions

All authors read and agreed to the published version of the manuscript. GFB and LAA: conceptualization of the manuscript and development of the methodology. GFB, BGY, FED, JCS, HT, AMJ, and LAA: writing the original draft of the manuscript. GFB, BGY, FED, JCS, HT, AMJ, and LAA: writing, review, and editing.

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