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Association of glyphosate with other agrochemicals: the knowledge synthesis[1]

Associação de glyphosate com outros agroquímicos: síntese do conhecimento

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Abstract - The association of agricultural chemicals complies with many objectives, such as the simultaneous control of organisms, dosage reduction, increase in the efficacy and prevention of cases of resilience to pesticides. The current paper aims to summarize information on the association of glyphosate with nutrients, herbicides and other pesticides. The association of glyphosate with polyvalent cations (e.g.: Ca and Mn), in general, reduces the efficacy of the herbicide, which can be overcome with the addition of ammonium sulfate to the application spray. The association of glyphosate with other herbicides depends on the used dosages, on the vegetable species, on the evaluation period, on the plant's development stage and on the biochemical compatibility between the action mechanisms of the herbicides. The association of glyphosate with systemic herbicides, in general, presents higher compatibility and benefits in contrast to the mixture with contact herbicides. The association of the glyphosate with auxin mimicking agents, in general, results in a synergetic effect. The mixture of glyphosate with ALS inhibitors may generate synergetic, additive, or antagonistic effects, presenting higher dependence on the doses of glyphosate on the mixture. There are many examples of antagonism among glyphosate and contact herbicides, such as inhibitors of GS, FSII, FSI and PROTOX. There is a lack of publications on glyphosate associated with fungicides or insecticides, and they do not prove synergetic or antagonistic effects of this mixture. However, papers that document the metabolization of glyphosate by plants suggest the need to investigate the impact of insecticides and fungicides in the action of the herbicide.
Keywords: synergism; antagonism; mode of action

Resumo - A associação de agroquímicos atende a muitos objetivos, como o controle simultâneo de organismos, reduções de doses, aumento da eficácia e prevenção de casos de resistência a pesticidas. O presente trabalho objetiva sintetizar informações sobre a associação de glyphosate com nutrientes, herbicidas e outros pesticidas. A associação de glyphosate com cátions polivalentes (ex: Ca e Mn) em geral reduz a eficiência do herbicida, que pode ser superada com a adição de sulfato de amônio à calda de aplicação. A associação de glyphosate com outros herbicidas é dependente das doses utilizadas, das espécies vegetais, da época de avaliação, do estádio de desenvolvimento da planta e da compatibilidade bioquímica entre os mecanismos de ação dos herbicidas. A associação de glyphosate com herbicidas sistêmicos em geral apresenta maior compatibilidade e benefícios, em contraste à sua mistura com herbicidas de contato. A

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associação de glyphosate com mimetizadores de auxina em geral resulta em efeito sinérgico. A mistura de glyphosate com inibidores da ALS pode gerar efeitos sinérgicos, aditivos ou antagônicos, apresentando maior dependência da dose de glyphosate na mistura. Existem muitos exemplos de antagonismo entre glyphosate e herbicidas de contato, como os inibidores da GS, FSII, FSI e PROTOX. São escassas as publicações de glyphosate associado com fungicidas ou inseticidas e as mesmas não evidenciam efeitos sinérgicos ou antagônicos dessa mistura. No entanto, trabalhos que documentam a metabolização de glyphosate por plantas sugerem a necessidade de investigação do impacto de inseticidas e fungicidas na ação do herbicida.
Palavras-chaves: sinergismo; antagonismo; mecanismo de ação

Introduction

The occurrence of pests, diseases and weeds reduces productivity of the agricultural crops in up to 90% (Oerke, 2006). Most of the time, there is simultaneous infestation of more than one pest in agricultural areas. This way, it is common practice to associate chemicals for the simultaneous control of these organisms (Gazziero, 2015). In addition to these benefits, the association of herbicides is recommended to reduce the dosage or to increase the efficacy and also to prevent the evolution of organisms resistant to their control agents (Jacquemin et al., 2009; Vidal et al., 2010; Lagator et al., 2013).

The association of herbicides can result in synergetic, antagonist or neutral effects (also known as additives). Many scientific papers employ the method proposed by Limpel-Colby (Colby, 1967), where the effects of each product are evaluated when applied in isolation, and the result of the association is mathematically estimated based on those results. Later on, the estimated result is compared to the result of the association effectively observed in plants. It is worth highlighting that this method is simple, it does not present high capacity to detect synergetic or antagonistic effects. A more robust method is the one that evaluates the effects of the associations through curves of response-doses of the herbicides applied in isolation and /or in mixture (Streibig et al., 1998; Nielsen et al., 2004; Kruse et al., 2006; Blouin et al., 2010). The graphic curves or isoboles represent points of several mixture combinations with the same effect, 50% for example (ED_{50}). If the observed points produced by the mixture deviate from a theoretical isobole of additivity (line joining the ED_{50} of both herbicides applied in isolation), the mixture is either more effective (synergism) or less effective (antagonism) than expected from the effects of herbicides applied in isolation (Streibig et al., 1998; Kruse et al., 2006).

Glyphosate is an herbicide that inhibits the activity of the enolpyruvylshikimate phosphate synthase (EPSPs) enzyme, which is present in the biosynthesis route of the amino acids phenylalanine, tyrosine and tryptophan (Padgette et al., 1991). This product is one of the most commercialized herbicides in the world, mainly because of the reduction of the value by the loss of patent (Woodburn, 2000) and by the use in transgenic crops resistant to the product. The lack of residual activity of glyphosate can lead to its use in many occasions during the crop cycle. Many farmers have opted to associate glyphosate with other agricultural chemicals to decrease the traffic of equipment and reduce production costs.

The type of information on the effect of the mixtures depends on the audience. For the pesticide manufacturing companies, it is necessary to know the synergetic interactions among the herbicides to subsidize the register and the distribution of pre-formulated mixtures. For the agricultural extension agent, it is important to know about the antagonisms in the associations between agricultural chemicals and thus guide the farmers on the problems resulting from it. The objectives of this literature review are to summarize the scientific information on the association of glyphosate with nutrients, with herbicides and others with pesticides.

Effect of the Ions and Micronutrients in the Water

Divalent cations (calcium, magnesium, manganese and zinc) present in the water containing glyphosate and micro nutrients added in the tank may antagonize the efficacy of the herbicide (Bernards et al., 2005; Mueller et al., 2006; Chahal et al., 2012). For example, a study with barley (*Hordeum vulgare* L.) as a model plant showed the effect of the quality of water on the glyphosate activity. When the water has 45 ppm of Ca^{2+} there is reduction in the efficacy of the glyphosate, when compared to the distilled water (O'Sullivan et al., 1981). Another study showed that the concentration of Ca^{2+} above 250 ppm harms the efficacy of glyphosate in the control of *Brachiaria platyphylla*, *Amaranthus palmeri*, *Ipomoea lacunosa* and *Cyperus esculentus*. The type of glyphosate formulation (ammonium, isopropylamine and potassium) and the addition of ammonium sulfate did not reduce the antagonism (Mueller et al., 2006).

The manganese micro nutrient (Mn) harms the efficacy of glyphosate in the control of *Chenopodium album*, *Setaria faberi* and *Abutilon theophrasti*. However, when Mn was formulated as ethylenediaminetetraacetate (Mn-EDTA) there was no reduction in the efficacy of glyphosate on the tested species. The order of addition of components in the spray tank did not alter the negative effect of manganese in the control of the referred species (Bernards et al., 2005). Other authors also confirmed that fertilizers solutions containing polyvalent cations harms the glyphosate efficacy (Chahal et al., 2012).

The presence of cations in the leaf surface coming from soil particles brought on by wind may antagonize the glyphosate action. There is also evidence that certain species, such as *Abutilon theophrasti*, have leaf glands specialized in secreting inorganic compounds, including calcium and magnesium ions (Hall et al., 2000).

The existing antagonism between positively loaded ions are attracted by the negative load of the glyphosate molecule, forming complexes glyphosate-salt, that make penetration in the leaves difficult. The association of Ca^{2+} or Mg^{2+} may happen in the carboxyl group and the phosphonate group of the glyphosate molecule (Thelen et al., 1995; Sekhon, 2003).

In situations in which the quality of water harms glyphosate efficacy by the elevated presence of cations (Ca^{2+}, Mg^{2+}, Na^{2+}, Zn^{2+}, etc.), ammonia sulfate can be used to solve the problem of formation of glyphosate-salt complexes. However, the ammonium sulfate must be dissolved in water before adding the glyphosate. This adjuvant precipitates those cations, avoiding chelating with glyphosate and allowing the absorption of herbicide through the cuticle (Schönherr e Schreiber, 2004). The NH_4^+ in the ammonium sulfate competes with the calcium for the formation of the complex with the glyphosate molecule (Thelen et al., 1995), but it also enables the absorption of glyphosate.

Association of Glyphosate with Other Herbicides

The synergism in the glyphosate mixture with several systemic herbicides is well-documented in the literature (Table 1). However, there is also evidence of antagonist associations when there is incompatibility among the action mechanisms of the components in mixture (Table 1). In this condition, the translocation of glyphosate is reduced resulting from the quick action of one of the herbicides in the mixture. The result of the association of glyphosate with other herbicides depends on the used dosages, on the vegetable species, on the evaluation period, and on the biochemical compatibility between the action mechanisms of the herbicides.

Table 1. Effects of glyphosate associations with other herbicides on the control efficacy.

Herbicide	Mode of action	Glyphosate interactions	References
2,4-D	Auxinic	(-) 0 (+)*	O'Sullivan and O'Donovan (1980); Robinson et al. (2012); Wehtje and Gilliam (2012)
2,4-DB	Auxinic	0	Culpepper et al. (2001)
dicamba	Auxinic	(-)	O'Sullivan and O'Donovan (1980)
fluroxypyr	Auxinic	(+)	Chorbadjian and Kogan (2002)
MCPA	Auxinic	(-)	O'Sullivan and O'Donovan (1980)
chlorimuron-ethyl	ALS inhibitor**	(-) 0 (+)	Norris et al. (2001); Nelson and Renner (2002); Ellis and Griffin (2003); Maciel et al. (2011)
cloransulam-methyl	ALS inhibitor	(-) 0 (+)	Norris et al. (2001); Maciel et al. (2011)
halosulfuron	ALS inhibitor	0	Nelson and Renner (2002)
imazethapyr	ALS inhibitor	(-) 0 (+)	Norris et al. (2001); Li et al. (2002); Maciel et al. (2011)
imazaquin	ALS inhibitor	(-) 0 (+)	Norris et al. (2001)
metsulfuron-methyl	ALS inhibitor	(+)	Kudsk and Mathiassen (2004)
pyrithiobac	ALS inhibitor	0	Nelson and Renner (2002)
rimsulfuron	ALS inhibitor	0	Nelson and Renner (2002)
glufosinate	GS inhibitor	(-) 0	Chuah et al. (2008); Bethke et al. (2013)
bromoxynil	FS II inhibitor	(-)	O'Sullivan and O'Donovan (1980)
simazine + atrazine	FS II inhibitor	(-)	Vidal et al. (2003)
atrazine	FS II inhibitor	0	Bradley et al. (2000)
carfentrazone-ethyl	PROTOX inhibitor	(-) 0 (+)	Werlang and Silva (2002)
fomesafen	PROTOX inhibitor	0	Ellis and Griffin (2003)
lactofen	PROTOX inhibitor	0	Ellis and Griffin (2003)
saflufenacil	PROTOX inhibitor	0	Eubank et al. (2013)
MSMA	Unknown	(-)	Burke et al. (2007)
diquat	FS I inhibitor	(-)	Wehtje et al. (2008)
clomazone	Carotenoid inhibitor	(-)	Vidal et al. (2010)

* 0 indicates the neutral effect (also known as additive); (-) indicates antagonist effect and (+) indicates synergetic effect; ** ALS = acetolactate synthase enzyme; GS = glutamine synthetase enzyme; FS II = photosystem II; FSI = photosystem I; PROTOX = protoporphyrinogen oxidase enzyme.

The herbicides that mimic auxin (dicamba, MCPA and 2, 4-D) have systemic action and do not present antagonism in the action of glyphosate in the final control of several vegetable species. For example, there was no harm in the efficacy of glyphosate associated to the auxin herbicides in the control of test plants such as *Triticumaestivum*, *Hordeum vulgare* and *Avena sativa*, when compared to the action of isolated glyphosate (O'Sullivan and O'Donovan, 1980). The control of *Ipomoea* spp. and *Digitaria sanguinalis* with glyphosate in the dosage of 560 g ha^{-1} a.e., isolated or associated with 2,4-DB, has shown to be synergetic. When glyphosate was used in higher dosages (840 and 1.120 g ha^{-1} a.e.), the mixture of auxinic did not interfere in the control of plants from these two species (Culpepper et al., 2001). Similarly, the glyphosate mixture with 2,4-D did not affect the control of *Abutilon theophrasti* and *Ambrosia trifida* (Robinson et al., 2012). The association of glyphosate (in the dosages of 720 and 1.440 g ha^{-1} a.e.) with herbicide fluroxypyr resulted in synergism in the control of *Malvaparviflora* (Chorbadjian and Kogan, 2002). Synergism was also observed in the association of glyphosate with 2,4-D in the control of *Toxico dendronradicans* (Wehtje and Gilliam, 2012).

Synergism is seen in the association of glyphosate with several herbicides that inhibit the acetolactate synthase enzyme (ALS) (Starke and Oliver, 1998; Norris et al., 2001; Li et al., 2002; Nelson and Renner, 2002; Ellis and Griffin, 2003; Kudsk and Mathiassen, 2004; Maciel et al., 2011). The absorption and translocation of glyphosate is stronger when associated with ALS inhibitors (Starke and Oliver, 1998). However, antagonism is also observed in the association of glyphosate with ALS inhibitor herbicides, mainly when the glyphosate dosage is limited (Norris et al., 2001; Li et al., 2002). For instance, in *Abutilon theophrasti*, the antagonism between glyphosate and imazethapyr was only seen up until the dosage of 630 g ha^{-1} a.e. of glyphosate and absent when applying 840 g ha^{-1} a.e. (Li et al., 2002).

Contact herbicides quickly destroy the leaf tissues and harm the absorption and translocation of glyphosate. As a consequence, it is possible to observe loss in the systemic action of glyphosate. The glutamine synthetase, glufosinate ammonium synthesis inhibitor herbicide, associated with glyphosate, reduced the efficacy in the control of *Abutilon theophrasti*, *Chenopodium album* and *Setaria faberi*, compared to the effect of the last herbicide applied in isolation (Bethke et al., 2013). Antagonist effects of glyphosate with glufosinate ammonium were also seen on *Eleusine indica* (Chuah et al., 2008).

The photosystem II inhibitor herbicides (FS II), when applied to the leaves, antagonize the glyphosate. For example, in *Sorghum bicolor*, the mixture of glyphosate with simazine + atrazine reduced the initial control in comparison with isolated glyphosate. The adsorption of glyphosate to colloids in the formulation of triazines would explain the antagonism and the increase in the dosage of glyphosate compensates, at least in part, the antagonism of the FS II inhibitors (Vidal et al., 2003).

Protoporphyrinogen oxidase enzyme inhibitors (PROTOX) reduce the absorption (Werlang and Silva, 2002) and translocation of glyphosate (Eubank et al., 2013), promoting the antagonism in the control of weeds. The antagonism in the association between glyphosate and PROTOX inhibitors depends on the species and herbicides dosage (Werlang and Silva, 2002; Ellis and Griffin, 2003; Eubank et al., 2013). For instance, there was antagonism of glyphosate (252 g ha^{-1} a.e.) + carfentrazone-ethyl (15 and 30 g ha^{-1} a.i.) in the control of the species *Eleusine indica*. However, there was neutral effect (additive) when glyphosate was used in the dosage of 720 g ha^{-1} a.e. The same products associated in the same dosages presented synergetic effect in the control of *Digitaria horizontalis* (Werlang and Silva, 2002).

Herbicides inhibitors of the electron flow in the photosystem I (FS I) also act in membrane and antagonize the action of glyphosate. In fact, the association of diquat (FS I inhibitor) with glyphosate harmed the translocation of the latter and, as a result, an elevated regrowth of *Phyllanthus tenellus* plants was seen compared to the treatment with glyphosate applied in isolation (Wehtje et al., 2008).

Finally, the MSMA organo-arsenical herbicide, which also impacts the leaf membranes, antagonized the action of glyphosate in *Brachiaria ramosa*, *Amaranthus palmerii* and *Amaranthus retroflexus*. The association with MSMA reduced the translocation of glyphosate in around 8% in the species of *B. ramose* and *A. palmerii* (Burke et al., 2007).

Association of Glyphosate with Other Insecticides and Fungicides

In scientific literature, the reports the show the effects of joint application of glyphosate with insecticides are limited

(Pankey et al., 2004; Scroggs et al., 2005; Petter et al., 2007; Soltani et al., 2012) or fungicides (Bradley and Sweets, 2008; Grichar and Prostko, 2009; Soltani et al., 2012). From the researched papers, only the association of glyphosate with lambda-cyhalothrin and fipronil insecticides showed antagonism in the control of weed *Sesbania exaltata*, when compared to the effect of glyphosate applied in isolation (Pankey et al., 2004).

At least two causes of this absence of interaction between glyphosate and other pesticides may be listed. First, most of the researches published include high dosages (>750 g ha^{-1} a.e.) of glyphosate, which could mask possible antagonism in the tested associations.

Second, the publications that analyzed the glyphosate association with fungicides and insecticides assumed that this herbicide was not metabolized in vegetables and did not cover a diversity of species that enabled to test the hypothesis of synergism. However, recent evidence shows that glyphosate is detoxified in *Mucuna pruriens* (Rojano-Delgado et al., 2012), *Conyza canadensis* (Gonzalez-Torralva et al., 2012) and in *Digitaria insularis* (Carvalho et al., 2012). Therefore, there is a lack of researches to analyze if whether the association of insecticides or fungicides with glyphosate would be synergetic in the vegetable species that are capable of detoxing the herbicide or products of its action mode.

Final Remarks

Glyphosate stands out as the most used product in worldwide agriculture. Thus, there are many opportunities for mixture of this product with other agriculture chemicals. This literature review indicates that the association of glyphosate with di-cations diluted in the application spray antagonize the herbicide activity. Systemic herbicides such as auxinic and ALS inhibitors tend to synergize the action of glyphosate because they favor its absorption and translocation by plants. In contrast, contact herbicides that act in the cell membranes in the vegetable leaves harm the absorption/translocation of glyphosate and, therefore, antagonize its activity. There is a lack of research on the impact of insecticides and fungicides in the action of glyphosate in plants with the capacity of detoxifying the herbicide. Most of publications on glyphosate associated with fungicides or insecticides do not show synergetic or antagonistic effects of the mixture.

Besides these biochemical and physiological interactions reviewed here, the physical and chemical characteristics of pesticides would also influence the interaction with glyphosate. It is illustrated with the octanol-water (K_{ow}) partition coefficient, where glyphosate stands out for presenting one of the most elevated hydrophilicities (K_{ow}=0,0017). However, it is speculated that this glyphosate characteristic does not strongly affect in the association with lipophilic products because the components in the glyphosate formulation mediate the interaction among the surfaces of the products in mixture.

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