Impacts of the control of weeds and herbicides applied to natural enemies¹

Impactos do controle de plantas daninhas e da aplicação de herbicidas em inimigos naturais

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Abstract - Weeds compete for different resources with crops and the management of these plants is necessary so that production losses can be minimized. On the other hand, the diversity of plant species which behave as weeds can positively contribute to the survival of many arthropods and natural enemies. The objective of this review was to systematize the scientific evidence on the impacts of control of weeds and herbicides applied to non-target insects such as predators and parasitoids of pests, which are beneficial for agriculture and present in different agricultural crops. In this sense, consequences of control of weeds on insects present in agroecosystems were reported, as well as the potential risks of widespread use of herbicides on crops. Finally, this review compiles the current state of knowledge on ecological relationships in agricultural systems, focusing sustainable weed management, coupled with an integrated pest management. **Keywords:** genetically modified crops; IPM; parasitoids; weeds

Resumo - As plantas daninhas competem por diferentes recursos com as culturas agrícolas, sendo o manejo dessas plantas necessário para que se possa minimizar perdas na produção. Por outro lado, a diversidade de espécies vegetais que se comportam como planta daninha pode contribuir de forma positiva para a sobrevivência de diversos artrópodes e inimigos naturais. O objetivo dessa revisão foi sistematizar as evidências científicas sobre os impactos do controle de plantas daninhas e da aplicação de herbicidas em insetos não alvos, como os predadores e parasitoides de pragas, benéficos para a agricultura, e presentes em diferentes culturas agrícolas. Neste sentido, foram reportadas consequências do controle das plantas daninhas em insetos presentes nos agroecossistemas, bem como, os potenciais riscos do uso generalizado de herbicidas em culturas agrícolas. Por fim, essa revisão compila o estado atual do conhecimento de relações ecológicas em sistemas agrícolas, tendo como foco o manejo sustentável de plantas daninhas, aliado ao manejo integrado de pragas.

Palavras-chaves: culturas transgênicas; MIP; parasitoides; plantas daninhas

Introduction

In agriculture, weed control is essential to avoid competition and harm to commercial cultures (Kuva et al., 2007; Albajes et al., 2009). The control of these plants is carried out by

different methods (manual, mechanical, chemical, among others), and the use of herbicides is the most common one, often less costly and in some cases selective to the culture (James, 2007). In recent years, weed control practices in Brazil have been highlighted with

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 \overline{a} ¹ Received for publication on 25/08/2015 and approved on 24/11/2015.

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the use of genetically modified organisms (GMOs) that are tolerant or resistant to herbicides and the adoption of no-tillage.

With the advancement of genetic engineering, herbicide-resistant agricultural varieties have became commonplace in the world agro-ecosystems. Crops such as soybeans, maize, cotton and rapeseed have the resources of this new genetic technology, with prospects of increasing new areas of cultivation over the next few years (Dill et al., 2008; Brooks e Barfoot, 2011). In Brazil, planting herbicideresistant and genetically modified (GM) agricultural species have legally occurred from the year 2003 and in recent years this technology has just increased (Monquero, 2005).

GM plants are also an important strategy for pest insect control, for insecticide toxins are constitutively expressed in them, controlling target pests during all phenological stages of development and particularly in the critical phase of crops. Thus, there is substantial reduction in insecticide use in GM crops (Reed et al., 2001). However, the use of herbicides is still needed in these areas for the desiccation of plants in no-tillage and weed control (Hough-Goldstein, 2004).

Natural enemies play an important role in controlling pests in GMO crops because they are able to control toxin-resistant insect populations. In addition, predators, parasitoids and entomopathogens help control the populations of insects that develop in the areas of shelter, necessary to long-term maintenance of the technology (Yang et al., 2014). Finally, the toxins used in GM plants are very specific and control few insect orders (Monquero, 2005), which increases the importance of natural enemies for reducing populations of non-target pests.

No-tillage is an agricultural practice adopted in Brazil from the 1970s in order to protect and conserve the soil against erosion and minimize negative environmental impacts from the use of the conventional cultivation system (Anghinoni, 2007). On the other hand, for the implementation and consolidation of no-tillage, there was an increased use of herbicides due to the replacement of conventional soil management practices such as plowing and harrowing (Gomes e Christoffoleti, 2008). Thus, indiscriminate or careless use of these technological tools (no-tillage with herbicideresistant GMOs) has caused a number of problems. Among these, a higher probability of weed-resistant biotypes selection, as well as negative impacts on non-target organisms (Christoffoleti et al., 2008; Menezes et al., 2012a,b; Galon et al., 2014).

The diversity of weeds in agricultural and forestry systems is critical to the survival of arthropods and natural enemies. These plants are a source of shelter, refuge, food and favorable microclimate for the development of different insects and breeding sites for predators and parasitoids (Steinbauer et al., 2006; Silva et al., 2010). However, weed control can alter the abundance and flora of different plant species, which can damage the associated arthropods (Landis et al., 2000; Albajes et al., 2009).

The objective of this review was to systematize the scientific evidence on the impacts of weed control and herbicide application on non-target insects such as predators and parasitoids of the pests, beneficial for agriculture, and present in different crops.

Natural Enemies and Their Important Role in Agroecosystems

The Integrated Pest Management (IPM, also known as Integrated Pest Control (IPC)) in agriculture consists of a support system of isolated or associated decisions for the adoption of pest control tactics, based on cost/benefit analysis that consider the impacts on farmers, society and the environment (Kogan, 1998). Biological pest control is one of the IPM options, and consists in the use of predators, parasitoids or entomopathogens to suppress pest populations (Soares et al., 2009a,b). The combination of biological and chemical control decreases the number of pesticide applications,

enabling greater savings and less negative impact on the environment (Carvalho et al., 2001; Fragoso et al., 2001). However, within the rules of IPM, the use of pesticides that are selective to natural enemies is advised (Medina et al., 2003).

Among the natural enemies, parasitoids and predators stand out. The use of parasitoids is advantageous due to the ability to control several key crop pests such as the ones in cotton, sugarcane, eucalyptus, potherbs, maize, soybean, tomato, and also stored grains (Parra e Zucchi, 2004; Soares et al., 2007, 2009a). Parasitoid species reproduce in various stages of their hosts, such as eggs, larvae/caterpillars, pupae or adult insects.

Parasitoids acquire the essential nutrients directly from the host hemolymph at the beginning of the immature development, feeding on tissues at the end of this stage. Initially the storage tissues and then the others, usually killing the host (Salvador e Cônsoli, 2008). During adult stage, the parasitoids' diet is exclusively acquired from the plants through nectar and pollen. These plants are generally distributed in agricultural areas and many of them are considered weeds in this ecosystem (Menezes et al., 2012b).

Predatory insects may or not be entomophagous generalists and can attack different prey species at the same time during their life cycle, causing impact on populations of various pests (Albajes e Alomar, 1999). These arthropods naturally occur in agricultural and forestry systems, effectively contributing to the population balance of phytophagous insects, especially defoliating caterpillars, providing reduction in the use of plant protection products and the consequent preservation of the environment (Menezes et al., 2013).

Predators can also benefit from spontaneous flora of agricultural areas for their survival and reproduction (Muruyama et al., 2002). Some predatory insects have a zoophytophagous habit and part of their diet is provided by plants. This includes pollen consumption for those with chewing mouthparts

and water and minerals for sucklings (Evangelista Jr. et al., 2004). Several reports show that the quality of the host plant can improve development, fertility, size, survival and population density of non-strictly zoophagous natural enemies (Awmack e Leather 2002; Evangelista et al., 2003, 2004). Moreover, zoophytofagy allows predator insects to survive in the field in conditions of prey shortage (Coll e Guershon 2002; De Clercq 2002).

Weed Plants are a Habitat for Natural Enemies in Agroecosystems

Weeds benefit different arthropods, providing habitat, additional sources of food (pollen and nectar), favorable microclimate for the development of natural enemies, parasitoids and other insects, shelter and refuge from predators and to overcome disturbances caused by agricultural practices (Jonsson et al., 2008; Silva et al., 2010). Natural enemies benefit from the resources available for these plants, which favors their efficiency as pest regulators (Landis et al., 2000).

The tritrophic interaction between weeds, arthropod pests and their natural enemies is frequent in agricultural systems, forming a complex web of food available to insects (Norris e Kogan, 2000, Gibson et al., 2006). However, this interaction is not yet well defined and understood (Kruess, 2003). Studies have been conducted on organic crops that conserve a variety of weeds in order to observe the effects of vegetation diversification over time on the insect community present in those plants (Clough et al., 2007).

In a survey on the incidence of predators of the genus *Orius* sp. Wolff (Heteroptera: Anthocoridae) in cultivated and invasive plants, the importance of weeds in maintaining this insect in different crops was noted. Species *O. insidiosus* Say, *O. thyestes* Herring, *O. perpunctatus* Reuter and others of this kind were found in the various crops sampled, but especially in the following weeds: black-jack

(*Bidens pilosa* L.), spiny amaranth (*Amaranthus* sp.), Tropical Mexican clover (*Parthenium hysterophorus* L.) and joyweed (*Alternanthera ficoidea* L.). These weeds serve as a habitat for these species, providing shelter, pollen and preys (Silveira et al., 2003). Predator *Podisus nigrispinus* Dallas (Hemiptera: Pentatomidae) has benefited from extra food supply provided by plants *Ageratum conyzoides* L*., Amaranthus hybridus* L*. and B. pilosa*. These weeds present in cotton favor colonization and maintenance of *P. nigrispinus* (Evangelista Jr. et al., 2003).

In inundative releases of predatory mite *Neoseiulus californicus* McGregor (Acari: Phytoseiidae), a natural enemy of pest *Panonychus ulmi* Koch (Acari: Tetranychidae) in apple orchards, it was found that the population of *N. californicus*, after the first year in combination with acaricides, reduced by 58% the spraying of these products. In subsequent years, control of pest mite was conducted solely with *N. californicus*. The authors highlighted that the weeds benefited *N. californicus* remaining in the apple orchard, promoting its migration into the apple trees in the years following its introduction (Monteiro et al., 2002).

Weeds *Richardia* sp., *A. conyzoides* L. *Sonchus oleraceus* L., *B. pilosa* and *Rumex* sp. have favored the occurrence of predatory mites *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae) and *N. californicus* in strawberry crops, indicating its importance in maintaining these natural enemies in the field (Ferla et al., 2007). Furthermore, there were several natural enemies of the psyllid of guava, *Triozoida* sp. (Hemiptera: Psyllidae), these being Chrysopidae, Syrphidae, Nabidae and Coccinellidae found in Poaceae and other weeds present in that culture (Barbosa et al., 2003).

In *Eucalyptus* plantations, maintaining native vegetation strips or conserving an understory have favored greater diversity of natural enemies, contributing to the nonoccurrence of pest outbreaks (Strauss, 2001; Stainbauer et al., 2006).

Compatibility Between Herbicides and Natural Enemies

Widespread applications of herbicides can cause changes in vegetation composition of crops tolerant to these products, reflecting in plant diversity decline (Heard et al., 2005; Culpepper, 2006). Lower plant diversity can affect the abundance of natural enemies and harm the natural pest control in agricultural or forestry crops (Hawes et al., 2009; Lundgren, 2009). Therefore, practices aiming at maintaining plant diversity to a level that will not threaten the development of agricultural crops must be properly studied. This diversity will contribute to maintain different trophic groups, promoting a complex interaction among all species that make up the landscape and, where possible, a balance between crops and other organisms in the agro-ecosystem.

Exclusive use of chemical control of weeds still occurs in rural, urban and domestic environments (Bonnet et al., 2008). The preference for the use of herbicides is due to ease of acquisition and application, less use of manpower and control efficiency. Brazil is the largest consumer of the world's agrochemicals and herbicides are the most marketed (Reis et al., 2009).

The IPM includes strategies such as monitoring pest populations, control decision rules based on their density plus the use of a series of associated management tactics, including biological control (Bernal, 2008). To optimize the IPM, one of the measures suggested is to preserve the populations of beneficial organisms, such as parasitoids and predators of phytophagous insects and mite (Schmuck et al., 1997). The use of agrochemicals that are selective to natural enemies, including herbicides, is one of the principles adopted in IPM (Medina et al., 2003; Bueno et al., 2008).

Herbicides can directly or indirectly cause deleterious effects to non-target arthropods (Dewar et al., 2000, Menezes et al., 2014). The weeds lower diversity can interfere

in the natural enemies population establishment and dynamics (Evangelista Jr. et al. 2003). Another impact on predators and parasitoids can be caused by chemicals present in herbicides. In this context, the toxicity of natural enemies by herbicides urges the need for selectivity studies. Tests for selectivity to non-target organisms have been carried out with commercial formulations of agrochemicals, since the same active ingredient may be in different commercial formulations and concentrations, which can cause different impacts on these organisms (Hassan et al., 2000).

Laboratory tests have been the most used methods to assess agrochemicals impacts on natural enemies (Croft, 1990). Often, laboratory methodologies standardized by international groups such as the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) (Hassan et al., 2000; Hassan e Abdelgader, 2001) are employed for the assessment of mortality or reduction in a beneficial ability, such as parasitism, for instance.

Using this methodology, Giolo et al. (2005) have evaluated the selectivity of different commercial formulations of glyphosate to parasitoid adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) eggs. Herbicides ZappTM Qi and RoundupTM WG were slightly harmful, while RoundupTM, PolarisTM, GlizTM 480 CS,
Glyphosate NortoxTM, Glyphosate 480 Glyphosate NortoxTM, Glyphosate 480 AgripecTM and Roundup TransorbTM decreased parasitism ranging from 80.40 to 88.19%, being moderately harmful. Stefanello Jr. et al. (2008) have classified herbicides GramoxoneTM 200 (0.30% paraquat dichloride) and Primestra GoldTM $(0.83\%$ atrazine + 0.65% Smetolachlor) as harmful to *T. pretiosum*, causing reduction of 99.95 and 99.36% in parasitism capacity, respectively. As for Leite et al. (2015), they have observed that GesaprimTM 500 Ciba Geisy (atrazine) was slightly harmful to *Trichogramma bennetti* Nagaraja e Nagarkatti and *T. bruni* Nagaraja, but harmless

to *T. demoraesi* Nagaraja, *T. galloi* Zucchi and *T. pretiosum.*

Carmo et al. (2009) have performed selectivity tests of different pesticides used in soybean crops to eggs parasitoid *Telenomus remus* Nixon (Hymenoptera: Scelionidae). Herbicides 2,4-D, S-metolachlor, flumioxazin, paraquat dichloride + diuron, paraquat dichloride and glyphosate (Roundup $TransorbTM$ were selective. Herbicides glyphosate + imazethapyr, clomazone, glyphosate (GlizTM), glyphosate (Roundup ReadyTM) were harmless to the larval stage and slightly harmful (class 2) to the pupal stage *T. remus*.

Bernard et al. (2010) have evaluated the effect of agrochemicals used in Australian vineyards on the predatory mite *Euseius victoriensis* Womersley e James (Acari: Phytoseiidae). Mortality rate after 48 hours, four and seven days was not significant when it was exposed to glyphosate at a concentration of 1.0 L/ha. The predator fertility rate was not compromised either.

Herbicides applied in maize crops have different selectivity to the predator *P. nigrispinus*. Menezes et al. (2012a) have evaluated the effect of atrazine, nicosulfuron and their mixture at the recommended commercial dose of products applied to eggs, in immature and adult stages of this insect. The outbreak of the *P. nigrispinus* eggs was reduced by herbicides atrazine, nicosulfuron and the mix, while the immature stages were affected only by herbicide atrazine and the mix (atrazine + nicosulfuron). Increased doses of atrazine, mesotrione, nicosulfuron and paraquat, at 2, 4 and 10 times the recommended commercial dose, reduced the predator insect survival in adult and nymph stages. However, herbicide mesotrione was slightly toxic to *P. nigrispinus*, while atrazine and paraquat were the most toxic ones (Camilo et al., 2012).

The parasitoid of pupae *Palmistichus elaeisis* Delvare e LaSalle (Hymenoptera: Eulophidae) showed sensitivity to herbicides used on eucalyptus and maize crops in their

survival and reproduction. Herbicides applied in eucalyptus such as glufosinate ammonium salt and oxyfluorfen reduced parasitism and the emergence of this hymenoptera and were considered toxic to females of this insect when in contact with pupae of contaminated *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). On the other hand, herbicides glyphosate and isoxaflutole were selective to *P. elaeisis*, resulting in a high number of adults emerged from pupae treated with these products (Menezes et al., 2012b). As for herbicides atrazine, paraquat and nicosulfuron, registered for use in maize, they were toxic to *P. elaeisis* and reduced parasitism and the emergence of this insect in treated pupae of *T. molitor*. Herbicide tembotrione was selective to *P. elaeisis* (Menezes et al., 2014).

Other studies on herbicide toxicology using methodologies different from IOBC's were also conducted. Bastos et al. (2006) have observed that herbicides clomazone and paraquat + diuron have moderately reduced parasitism, development and emergence of *T. pretiosum* in eggs of *Sitotroga cerealella* (Olivier) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Addison e Barker (2006) have tested the effect of pesticides used in pastures on parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae). This one was introduced in New Zealand to control Argentine stem weevil *Listronotus bonariensis* **Kuschel** (Coleoptera: Curculionidae), the biggest pest of grasses in the region (McNeill et al., 2002 a,b). Herbicides asulam, metsulfuron, paraquat, and glyphosate were not toxic but surfactant Silwett L-77 has caused a high mortality rate in *M. hyperodae.*

Reviews of herbicidal effect on arthropods in the field were carried out by Albajes et al. (2009), observing changes in the composition and abundance of weeds in plots of herbicide-tolerant maize treated with glyphosate, affecting several groups of arthropods. Leafhoppers and aphids were more abundant in plots treated with herbicide, and the opposite occurred with thrips. Among predators,

the ones from the Araneae order and Trombididae family, besides *Orius spp*., were more abundant in the treated plots. As for *Nabis spp*. and carabids, they were more abundant in the untreated plots. Among the parasitoids, the Ichneumonidae order was more abundant in the untreated plots and Mymaridae in the treated plots.

McCravy e Berisford (2001) have determined whether the use of herbicides hexazinone, sulfometuron methyl, imazapyr and glyphosate in a *Pinus taeda* L. plantation in the state of Georgia, USA, affects parasitism of pest *Rhyacionia frustrana* (Comstock) (Lepidoptera: Tortricidae). The parasitism rate did not differ between plots treated with herbicides and untreated ones.

Herbicides that had the selectivity tested for natural enemies in agricultural and forestry systems were compiled in Table 1 and classified according to the impacts.

The process of registering an agrochemical in Brazil is regulated by Act No. 7802 of 1989 by its regulatory Ruling 4074 and normalizing ordinances. It is based on acute and chronic toxicological tests with vertebrates (mammals) and acute toxicology with nontarget organisms (in these tests soil microorganisms, algae, earthworms, bees, microcrustaceans, and fish are represented). Studies of selectivity and chronic effects on natural enemies of ecosystems to which the products will be released are not required in Brazil, and these studies are from universities and research institutions. This differs from the protocol adopted in Europe since the 1990s, where a dossier testing the influence of agrochemicals on beneficial organisms on site is required for product registration in several countries. To do so, Europe has approved methodologies of international analysis using standard methods that allow comparing and contrasting results from one country to another. Such studies may not be suitable to evaluate the use of a product in the IPM, but allow estimating the potential impact to natural enemies and

provide information about the correct way to implement them (Olszak et al., 1999).

Table 1. Herbicide selectivity (commercial product or active ingredient) for different arthropods used in the biological control of agricultural pests.

Herbicides*			
Insects	Selective	Non-Selective	Author
Trichogramma pretiosum	Zapp™ Qi and Roundup™ WG	Roundup™, Polaris [™] , Gliz [™] 480 CS, Glyphosate Nortox TM , Glyphosate 480 Agripec™ and Roundup TransorbTM	Giolo et al. (2005)
T. pretiosum		Gramoxone 200 (0.30% paraquat dichloride) and Primestra Gold $(0.83\% \text{ atrazine} + 0.65\% \text{ S} -$ metolachlor),	Stefanello Jr. et al. (2008)
T. pretiosum		clomazone and paraquat $+$ diuron	Bastos et al. (2006)
Trichogramma bennetti and T. bruni		Gesaprim 500 Ciba Geisy (atrazine)	Leite et al. (2015)
Trichogramma demoraesi, T. galloi and T. pretiosum.	Gesaprim 500 Ciba Geisy (atrazine)		Leite et al. (2015)
Telenomus remus	2,4-D, S-metolachlor, flumioxazin, paraquat dichloride + diuron, paraquat dichloride and glyphosate (Roundup TransorbTM) glyphosate + imazethapyr, clomazone, glyphosate (GlizTM), Glyphosate (Roundup ReadyTM) (larval stage)	glyphosate + imazethapyr, clomazone, glyphosate (GlizTM), glyphosate (Roundup ReadyTM) (pupal stage)	Carmo et al. (2009)
Euseius victoriensis	glyphosate		Bernard et al. (2010)
Podisus nigrispinus		atrazine, nicosulfuron and mix (atrazine + nicosulfuron) (eggs hatching) atrazine and mix (atrazine + nicosulfuron) (unripe stage)	Menezes et al. (2012a)
P. nigrispinus	mesotrione	Atrazine and paraquat	Camilo et al. (2012)
Palmistichus elaeisis	glyphosate and isoxaflutole	glyphosate ammonium salt and oxyfluorfen	Menezes et al. (2012b)
	tembotrione	atrazine, paraquat and nicosulforon	Menezes et al. (2014)
Microctonus hyperodae	asulam, metsulfuron, paraquat, and Glyphosate	surfactant Silwett L-77	McNeill et al. (2002 a,b)
Orius spp.	glyphosate		Albajes et al.
Nabis spp.		glyphosate	(2009)
Rhyacionia frustrana		hexazinone, sulfometuron methyl, imazapyr and glyphosate	McCravy e Berisford (2001)

*As a criterion to define herbicide selectivity, products lightly and moderately harmful to non-target organisms were considered as non-selective.

The studies analyzed in this review reported various toxicities of herbicides on predators and parasitoids and raised hypotheses to explain toxic effects. Several factors were identified as causing this toxicity, including high doses, amount and type of active ingredients present, salts and adjuvants in the formulation and synergistic effects of mixing products. The most indicated routes of contamination of the natural enemies were the ingestion of herbicides (directly from the spray droplet or through the plant or contaminated prey/host) or even by direct penetration of the product into the insect's body through the cuticle after spraying. However, the mechanisms and physiological routes by which herbicides can poison the natural enemies remain unknown to science.

Final Remarks

The Sustainable Weed Management allied with the practices of Integrated Pest Management contributes with a new insight into current and future agriculture cultivations in Brazil, relating the ecology of the agricultural environment with the commercial culture itself. Weed management contributes to the maintenance of different arthropods, predators and parasitoids that are essential for the natural control of various agricultural pests.

Genetically modified agricultural crops have satisfactorily settled in the world market and in Brazil, with prospects of increasing in production areas. However, the widespread use of herbicides on crops can cause negative impacts on the environment such as the selection of resistant weed species or natural enemies poisoning. Furthermore, studies show that some chemical products are selective to different non-target organisms and safe for use in the environment. In an overview, it is necessary that users of these products do not use them indiscriminately or negligently. Therefore, more studies must be performed in order to know these negative impacts and enable plant diversity in these agroecosystems so that they favor natural enemies and do not harm the crops.

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