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Occurrence and importance of herbicide resistance caused by degradation enhancement for weed management[1]

Ocorrência e importância da resistência a herbicidas causada por incremento de metabolização para o manejo de plantas daninhas

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Abstract - Non-target-site resistance (NTSR) to herbicides mainly caused by enhanced degradation is highly problematic due to the occurrence of biotypes with multiple resistance and is a new challenge for weed management and herbicide use. Recently, enzymes associated with xenobiotic degradation especially cytochrome P450, GSTs and ABC transporters have been associated with the herbicide resistance in several weeds. This knowledge opens a new window to understand the evolution of NTSR. The aims of this review are to discuss the current knowledge of the gene regulation associated with the herbicide resistance caused by enhanced herbicide degradation and to analyze the main consequences of this problem for the adequate herbicide use and weed management. Multiple herbicide resistance caused by degradation enhancement occurs in *Lolium rigidum*, *Alopecurus myosuroides*, *Echinochloa phyllopogon* and in several other species. The level of herbicide resistance caused by degradation enhancement is affected by environmental factors in several cases, which difficult the resistance diagnostic and facilitate its distribution. The modern weed management should consider the characteristics of the herbicide degradation, since the simple rotation of herbicides mechanism of action might not be enough to prevent the herbicide resistance. The use of synergistic mixtures of herbicides and other enzyme inhibitors may contribute to prevent the evolution and spreading of NTSR weed herbicide resistance.
Keywords: multiple resistance; cytochrome P450; detoxification; NTSR

Resumo - A resistência a herbicidas causada por mecanismos não relacionados ao local de ação (NRLA), principalmente causada pelo incremento de metabolização, é altamente problemática devido à ocorrência de biótipos com resistência múltipla, e é um novo desafio para o manejo de plantas daninhas e utilização de herbicidas. Recentemente, enzimas associadas à degradação de xenobióticos, especialmente citocromo P450, GSTs e transportadores ABC, têm sido associadas à resistência a herbicidas em várias plantas daninhas. Os objetivos desta revisão são descrever o

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conhecimento atual da regulação gênica associada à resistência a herbicidas causada pelo incremento de metabolização e analisar as principais implicações deste problema para definir estratégias adequadas de manejo com o uso de herbicidas. A resistência múltipla a herbicidas causada pelo incremento da degradação ocorre em *Lolium rigidum*, *Alopecurus myosuroides*, *Echinochloa phyllopogon* e outras várias espécies. O nível de resistência a herbicidas causada pelo incremento de metabolização é afetado por fatores ambientais em vários casos, o que dificulta o diagnóstico da resistência e facilita sua distribuição. O manejo moderno de plantas daninhas deve considerar as características de degradação do herbicida, uma vez que a simples rotação de mecanismo de ação de herbicidas pode não ser suficiente para prevenir a ocorrência da resistência a estes produtos. A utilização de misturas sinergísticas de herbicidas e outros inibidores enzimáticos pode contribuir para prevenir a evolução e distribuição da NRLA em plantas daninhas.
Palavras-chaves: resistência múltipla; citocromo P450; detoxificação; resistência NRLA

Introduction

The weed herbicide resistance is a problem that is challenging not only the weed control but also crop management worldwide. There are two primary mechanisms of resistance: resistance related to the herbicide target site of action (TSR) and non-target-site resistance (NTSR) (Yuan et al., 2007). TSR is caused mainly by mutation that change an amino acid causing alteration in the enzyme conformation, preventing the binding of the herbicide in the site of action (Powles and Yu, 2010). In addition, TSR herbicide resistance can be caused by the increasing in the expression of the target enzyme, which can occur due to the amplification of the gene and mutations in the promoter region (Gaines et al., 2010; Powles and Yu, 2010). NTSR occurs due to mechanisms that limit the arrival of the lethal dose of the herbicide to the site of action. These mechanisms include mainly lower absorption, alteration in translocation and degradation enhancement (Powles and Yu, 2010).

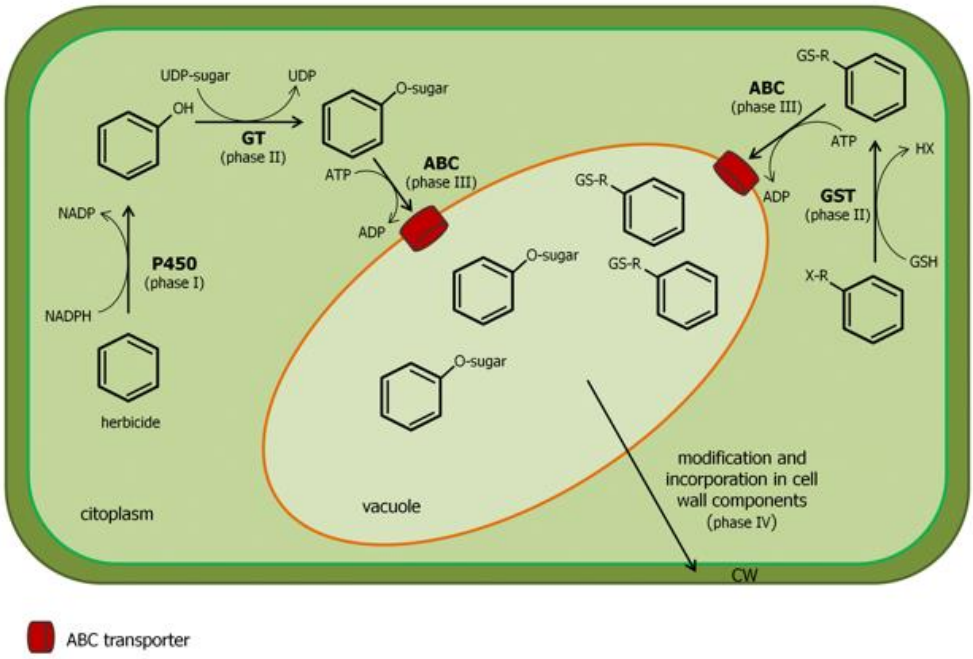
The enhanced herbicide degradation is caused by increasing activity of detoxifying enzymes, which transforms the herbicide into less toxic compounds than the parent molecule. Similar detoxification processes associated with NTSR present in weeds are also related with the natural herbicide tolerance in several crops (Devine et al., 1993). The main enzymes involved in this process are cytochrome P450 monooxygenases (CytP450) and glutathione S-transferase (GST) (Powles and Yu, 2010). This type of resistance is involved in multiple resistance phenomena, in which plants are resistant to herbicides of several chemical families belonging to different herbicide mechanisms of action (Buono and Ioli, 2011). Therefore, NTSR usually is associated with complex pattern of cross and multiple resistance that decrease the herbicide options for controlling the resistant populations.

The objectives of this review are to discuss the current knowledge of the gene regulation associated with herbicide resistance caused by enhanced degradation and to analyze the main consequences of this problem for the adequate herbicide use and weed management.

Phases of Detoxification

The detoxification of herbicides in plants is divided into four phases (Yuan et al., 2007): phase I - conversion; phase II - conjugation; phase III - secondary conversion and transportation for the vacuole and; phase IV - final metabolite deposition (Figure 1). During phase I, the herbicide molecule goes through chemical changes such as oxidation, reduction, hydrolysis, oxygenation or hydroxylation. In this process, the main players are CytP450 enzymes, which catalyze reactions of oxidation and reduction of endogenous substrates and xenobiotics (Yuan et al., 2007). In this phase, the enzyme responsible for the hydrolysis of the herbicide molecule - aryl acylamidase - can also

act (Powles and Shaner, 2001). On phase II, the herbicide molecule or metabolite resulting from phase I is conjugated with hydrophobic or electrophilic substrates (sugars, amino acids or glutathione) by enzymes from the GST family or by glycosyltransferase (GTs) (Reade et al., 2004, Yuan et al., 2007). This reaction increase the water solubility of the molecule and decrease herbicide phytotoxicity (Carvalho et al., 2009). On phase III, the metabolites resulting from phase II are transported to the vacuole by ABC transporters (ATP-binding cassete) (Yuan et al., 2007) (Figure 1). In this phase, secondary conjugation can also occurs, generating non-phytotoxic compounds (Hatzios, 1991). Finally, on phase IV, the metabolite(s) from the detoxifying process compartmentalized in the vacuoles can be associated with the components of the cell wall, such as pectin, lignin, polysaccharides and protein fractions, forming insoluble residues (Skidmore, 2000).



(Phase I), reaction of conjugation of sugars or glutathione (GSH) by glycosyltransferase enzymes (GTs) or glutathione S-transferases (GSTs), respectively (phase II), transportation via ABC transporters, followed by compartmentalization in vacuole (phase III) and incorporation in the cell wall (CW) (phase IV).

Figure 1. Representation of enzyme activity in the detoxification of herbicides in plants. Adapted from Yu and Powles (2014).

Enzymes Involved in the Herbicide Degradation

Cytochrome P450 Monooxygenases - Phase I of Detoxification

The P450 cytochrome gene family (CytP450) is among the most abundant in plants (Nelson and Werck-Reichhart, 2011). P450 enzymes are known as key-enzymes on phase I of the metabolism of xenobiotics, and they have a crucial role in detoxification of herbicides in plants (Yun et al., 2005). They are hemeproteins that usually catalyze reactions of monooxygenation dependent on oxygen and NADPH (Yuan et al., 2007). These enzymes are involved in the fast evolution of herbicide resistance in ryegrass (*Lolium rigidum*), especially due to the use of subdoses of herbicides (Yu et al., 2013; Busi et al., 2013). In *Echinochloa phyllopogon* higher expression of *CytP450* genes *CYP81A12* and *CYP81A21* were found in plants resistant to herbicides

bensulfuron-methyl and penoxulam, both inhibitors of enzyme acetolactate synthase (ALS) (Iwakami et al., 2014a). In the same species, genes *CYP71AK2* and *CYP72A254* had greater expression in plants resistant to herbicide bispyribac-sodium (Iwakami et al., 2014b). In *Echinichloa crus-galli*, the gene *CYP81A6* had greater expression in plants resistant to herbicide imazethapyr (Dalazen et al., 2015b).

In addition to being responsible for the resistance in weeds, genes *CytP450* are also related to the herbicides tolerance mechanisms in crops, such as *CYP72A21* and *CYP81A6* in *O. sativa* (Liu et al., 2012; Pan et al., 2006; Hirose et al., 2007), *CYP72A5* in *Zea mays* (Persans et al., 2001), *CYP81B2* and *CYP71A11* in *Nicotiana tabaccum* (Yamada et al., 2000), *CYP71C6v1* in *Triticum aestivum* (Xiang et al., 2006) and *CYP71A10* in *Glycine max* (Siminszky et al., 1999).

Aryl Acylamidases - Phase I of Detoxification

Aryl acylamidases are enzymes that catalyze the hydrolysis of certain acylamidases (Powles and Shaner, 2001). Detoxification of the herbicide propanil by the action of these enzymes in rice leads to the formation of 3,4-dichloroaniline (DCA) and propionic acid. Studies carried out with biotypes of *Echinochloa* spp. resistant to propanil also indicate the participation of this enzyme in detoxification (Hoagland et al., 2004). In a biotype of *Leptochloa chinensis* resistant to propanil was identified the presence of DCA after herbicide application and increase in the activity of enzyme aryl acylamidase in comparison with the susceptible biotype (Ismail et al., 2013).

Glutathione S-transferases (GSTs) - Phase II of Detoxification

Glutathione S-transferases (GSTs) are enzymes that act on phase II of detoxification, catalyzing the conjugation of the thiol group of glutathione (GSH) with electrophilic centers of lipophilic molecules to form less active products (Edwards et al., 2011; Öztetik, 2008). These enzymes may be induced by biotic or abiotic stresses, such as osmotic stress and high temperatures, in addition to oxidative stress caused by herbicides (Dixon et al., 2002).

Detoxification by conjugation with glutathione (GSH) is mainly observed for herbicides belonging to the group of chlorotriazine, diphenylethers, chloroacetanilides, sulfonylureas and aryloxyphenoxypropionate (Cummins et al., 2011), especially in poaceae. Plants of *E. crus-galli* resistant to quizalofop-p-ethyl had more activity of a GST when compared to the susceptible biotypes (Huan et al., 2011). In resistant biotypes of *E. crus-galli*, the gene *EcGST1* that encodes a GST changed the expression level from 6 to 10-fold after aspersion of quinclorac herbicide (Li et al., 2013). Some studies indicate that resistance of *Alopecurus myosuroides* to several herbicides is caused by a GST coded by gene *AmGSTF1* (Cummins et al., 2013). The gene *OsGSTL2* is responsible by encoding a GST, which is involved in the detoxification of herbicide chlorsulfuron in *O. sativa*. The overexpression of this gene in plants of rice showed an increase in the tolerance to chlorsulfuron and glyphosate (Hu, 2014).

Despite the participation of GSTs in the detoxification of herbicides in poaceae, there is also information on the activity of these enzymes in plants of other botanic families. The detoxification of atrazine in resistant plants of *Abutilon theophrasti* is related to the action of GSTs (Gronwald and Plaisance, 1999). Plants of *Sonchus oleraceus* resistant to simazine, when exposed to this herbicide, showed a GST activity of around 8.3 times in relation to the susceptible (Fraga and Tasende, 2003). GST enzymes are also involved in the tolerance of crops to herbicides, such as the detoxification of chloroacetanilides and diphenylethers in soybean by the enzyme coded by the gene *GmGSTU4* (Benekos et al., 2010).

Glycosyltransferase (GTs) - Phase II of Detoxification

Glycosyltransferase (GTs) are enzymes that catalyze the glycosidic binding between sugar and an acceptor, which can be a range of biomolecules, including other sugars, proteins, lipids and small molecules (Gloster, 2014). GTs families are able to recognize hormones and secondary metabolites, as well as natural toxins and chemicals (Bowles and Lim, 2010).

The action of GTs in the direct conjugation of herbicides in plants was observed in 2,4-D, clopyralid, chloramben, diclofop-methyl, maleic hydrazide, MCPA, metamitron, metribuzin, picloram and quinclorac. However, conjugation of herbicides 2,4-D, acifluorfen, bentazon, dicamba, diclofop-methyl, methyl-flamprop, fluorodifen, sulfonylureas and propanil, in some plant species, was only possible after activation by cytP450 enzymes (Schröder and Collins, 2002). In some cases, direct conjugation of certain herbicides (e.g. 2,4-D and diclofop-methyl), mediated by GTs, does not result in stable detoxification in the plant. This happens due to the fast conversion of the conjugated to the active form of the herbicide (Kreuz et al., 1996).

Differently from what is observed in the cytP450 and GSTs enzymes, there are few studies that show the action of GTs as the main resistance mechanism of weeds to herbicides. In a study with *A. myosuroides* was detected higher activity of GTs in the resistant population comparing to the susceptible one (Brazier-Hicks et al., 2002). Nonetheless, resistance of this population was also associated to the action of cytP450 and GSTs enzymes.

ABC Transporters (ATP- Binding Cassete) - Phase III of Detoxification

ABC transporters correspond to a superfamily of transporters bound to the membrane and soluble proteins that transport the molecules through the cellular membrane of a process that involves the hydrolysis of ATP (Remy and Duque, 2014). In most cases, transport involves the removal of cytosol substrates for extra cytosolic compartments, including extracellular space or vacuole (Frelet-Barrand et al., 2008).

In plants, there are reports of ABC transporters acting in the detoxification of herbicides, more specifically in the transportation of S-glutathionylation conjugates. The induction of *ZmMRP1* expression was seen in plants of corn after the application of selective herbicides 2,4-D, atrazine, metolachlor and primisulfuron (Pang et al., 2012). In *Arabidopsis thaliana*, the overexpression of gene *AtPgp1* increased the resistance to herbicides dicamba, pendimethalin, oryzalin and MSMA (Windsor et al., 2003). The loss of function of gene *AtPDR11* in plants of *A. thaliana* gave increased the paraquat tolerance (Xi et al., 2012). Recently, the resistance of *Conyza canadensis* to glyphosate was associated to the synchronization of the greater expression of genes that encode the EPSPS enzyme and ABC transporters (Tani et al., 2015). M10 and M11 genes had higher expression (four times) in plants treated with glyphosate 24 hours after the application of the herbicide, with significant reduction in the expression level of these genes after this period.

Detection of Resistance by Increase of Detoxification and Related Factors

Enzyme Inhibitors

The enhanced degradation of herbicides caused by cytP450 enzymes can be indirectly identified through enzyme inhibitors, such as 1-aminobenzotriazole (ABT), piperonyl butoxide (PBO) and malathion (Elmore et al., 2015; Siminszky, 2006). The insecticide organophosphate malathion is one of the most commonly used inhibitor in this kind of evaluation (Yasour et al., 2009, Matzenbacher et al., 2015). The inhibition occurs when the sulfur atom released by the organophosphate oxygenation inhibits the action of the cytP450 enzyme (Werck-Reichhart et al., 2000). In *L. rigidum*, the use of malathion reversed the

resistance to chlorsulfuron (Yu et al., 2009). Similarly, in *E. phyllopogon*, the use of cytP450 malathion inhibitor increased the effect of the herbicide penoxsulam in resistant plants due to decreasing the herbicide detoxification (Yasour et al., 2009). In a biotype of *E. crus-galli* resistant to herbicide imazethapyr, resistance was partially reversed by malathion and PBO, both applied in the doses of 1000 g ha^{-1}, two hours before the application of the herbicide (Dalazen et al., 2015a). In this study, resistance factor was reduced from 15.94 to 3.44 and 4.94, respectively, with the application of malathion and PBO.

Inhibition of enzyme aryl acylamidase can also occur with the application cytP450 inhibitors. The resistance of *E. crus-galli* and *E. colona* to herbicide propanil was decreased with application of 1100 g ha^{-1} of carbaril at 24 hours before the herbicide (Hoagland et al., 2004).

The herbicide resistance associated to GSTs enzymes can be detected through compound that mimic glutathione (GSH). These compounds bind to the site of conjugation of the GST enzyme, inhibiting the binding of the herbicide or a metabolite from phase I of detoxification (Cummins et al., 2013). The GST inhibitor 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl), applied in the rate of 270 g at ha^{-1} 48 hours before the application of herbicides fenoxaprop-p-ethyl and clodinafop-propargyl increase the control of *A. myosuroides*, resistant to these herbicides (Cummins et al., 2013). Tridiphane also acts as a GST inhibitor, and reversed the resistance of *Lupinus angustifolius* to metribuzin, applied in the dosage of 50 g ha^{-1}, two hours before the herbicide application (Pan et al., 2012).

Differential Gene Expression and Epigenetic Regulation

Recently, several molecular biology studies have helped the identification of the mechanisms of herbicide resistance in different weeds (Délye et al., 2015). The evaluation of the differential gene expression can be done through reactions of qRT-PCR (*Real time Quantitative Polymerase Chain Reaction*). This technique is a versatile analytical tool in which a fluorophore is used to report the amount of PCR product in real time for each PCR cycle, as an indicative of gene expression (Délye et al., 2015). qRT-PCR was used in plants of *E. phyllopogon* for the evaluation of differential gene expression of the cytP450 genes *CYP81A12* and *CYP81A21* (Iwakami et al., 2014a) and *CYP71AK2* and *CYP72A254* (Iwakami et al., 2014b), which were higher expressed in resistant biotypes comparing to susceptible ones. In *E. crus-galli* higher expression of gene *EcGST1* (Li et al., 2013) and *CYP81A6* (Dalazen et al., 2015b) was associated with herbicide resistance. Similarly, the GST gene *AmGSTF1* was related with the herbicide resistance in *A. myosuroides* based on gene expression studies carried out by qRT-PCR (Cummins et al., 2013).

In addition to DNA mutations or indels in the target genes or genes associated with the herbicide translocation or degradation, the herbicide resistance can also be caused by epigenetic factors. Recently, some authors have suggested that epigenetic regulation can be involved in the evolution of resistance of weeds to herbicides (Gressel, 2009; Délye, 2013). The hypothesis of involvement of the epigenetic regulation in the evolution of resistance is based on the fact that some herbicides can cause oxidative stress similar to those caused by abiotic stresses in plants (Eldeen and Radwan, 2012). Many enzymes related with stress response pathways are also important to herbicide detoxification, such as reactive oxygen species (ROS) related enzymes (Tausz, 2001). Thus far, there are a few studies that associated herbicides effects and with epigenetic regulation, as well as the consequences on the gene expression related to herbicide detoxification or any other mechanism of herbicide resistance.

In a study carried out with glyphosate in *T. aestivum*, different herbicide concentrations changed levels of DNA methylation, ranging from 28.3 to 73.9% (DNA hypermethylation)

(Nardemir et al., 2015). Preliminary studies in *A. thaliana* indicated that herbicides imazethapyr, glyphosate and 2,4-D did not induce alterations on global DNA methylation (Markus, non-published data). However, the evaluation of different epigenetic mutants of *A. thaliana* showed higher herbicide susceptibility for some mutants indicating that epigenetic regulation may be associated with the herbicide effect (Markus, non-published data). Therefore, the epigenetic regulation trigged by abiotic stresses in interaction with the herbicide effect should be considered in studies of herbicide resistance, although the confirmation of this phenomenon is still in development.

Activity of Detoxifying Enzymes

The enhanced herbicide degradation is caused by the higher activity of detoxifying enzymes (Yu and Powles, 2014). In resistant plants these enzymes have greater metabolic activity and, consequently, higher consumption of substrate (herbicide). Therefore, the evaluation of the detoxifying enzymes activity, such as cytP450 and GSTs, can be an indication of herbicide resistance caused by enhanced degradation (Yu et al., 2005).

In a study with *E. crusgalli*, the biotypes resistant to ACCase herbicides had greater activity of the GST enzyme in comparison with the susceptible biotypes (Huan et al., 2011). In another study, the activity of cytP450 enzymes was evaluated in resistant and susceptible biotypes of *E. phyllopogon* (Yun et al., 2005). The resistant plants had greater concentration and activity of cytP450 enzymes, especially in the herbicide treated plants.

The activity of herbicides detoxifying enzyme as well as the other enzymes depends on environment factors, mainly temperature (Mahan et al., 2004). Some studies have shown the relationship between temperature and herbicides detoxification due to the activity of detoxifying enzymes. In *Amaranthus palmeri*, both detoxification rate and the amount of detoxified mesotrione were higher under higher temperatures (40/30°C - day/night) in relation to plants grown in lower temperatures (25/15°C and 32,5/22,5°C) (Godar et al., 2015). Similarly, several grass weeds had, on average, 56% and 68% of detoxification of amicarbazone under temperatures of 25/20°C and 40/35°C, respectively (Yu et al., 2015). Although the enzyme activity has not been measured in both studies, the authors attribute the higher detoxification of the herbicide under higher temperatures to the high activity of detoxifying enzyme. Nevertheless, some studies confirm higher activity of detoxifying enzymes in high temperatures. In plants of *A. myosuroides*, the GST enzyme activity was higher in plants grown at 25°C in comparison with 10°C, especially in the resistant biotype (Milner et al., 2007).

Analysis of the Concentration of Herbicides and their Metabolites

The presence of variable amounts of herbicide in tissues of treated plants, as well as the detection of their metabolites, is an indication of herbicide detoxification. The degradation enhancement of fenoxaprop and mesosulfuron+iodosulfuron in plants with multiple resistance was evaluated in *A. myosuroides*, through an analysis by LC/MS-MS (*Liquid Chromatography/tandem Mass Spectometry*) (Kaiser and Gerhards, 2015). In resistant plants, the amount of herbicides analyzed was smaller in comparison to the susceptible plants.

High-performance liquid chromatography (HPLC) analysis has also been used in herbicide resistance mainly studies (Yu and Powles, 2014). In this approach, the detection of herbicide in the parental form and its metabolites is done through the metabolic profile analysis of the radioactively marked molecules mainly with [^{14}C]. In plants of *L. rigidum*, the metabolic profile of the herbicide [^{14}C] chlorsulfuron indicated the presence of herbicide in its parental form in susceptible plants, showing that there is no detoxification in this biotype. However, in the resistant biotype, the amount of herbicide detected in the parent

form was smaller and a large amount of herbicide metabolite was identified (Christopher et al., 1991). The same methodology was used in the analysis of glyphosate and its metabolites in resistant and susceptible plants of *Digitaria insularis* (Carvalho et al., 2013) and *C. canadensis* (González-Torralva et al., 2012). In resistant biotypes, the glyphosate degradation in the metabolites aminomethylphosphonic acid, glyoxylate and sarcosine was higher than in the susceptible biotype.

Importance of Enhanced Degradation Mechanism of Resistance for Weed Management

Cross and multiple weed herbicide resistance has become increasingly frequent, showing the action of complex resistance mechanisms. The enhanced herbicide degradation is involved in the multiple resistance phenomena, in which plants are resistant to herbicides of several chemical families belonging to different herbicide mechanisms of action (Buono and Ioli, 2011). Herbicide resistance in *L. rigidum* is one of the most impacting example of multiple herbicide resistance evolution. In this species, at the same time there are several populations with TSR and NTSR the same individual common (Powles and Yu, 2010; Han et al., 2014). In Australia, six populations of *L. rigidum* are described for having crossed or multiple resistances, in which there is herbicide resistance to ACCase, ALS, photosystem II and dinitroanilines (Yu and Powels, 2014). The population VLR69 of *L. rigidum*, after 21 years of selection by several herbicides, presented resistance to 11 groups of herbicides belonging to five action mechanisms (Heap, 2014).

The increasing number of multiple resistance cases due to enhanced herbicide degradation must be considered as a serious threat for the efficacy of herbicides (Délye, 2013; Yu and Powles, 2014). The management strategies for herbicide resistance prevention based on the rotation of herbicides with different mechanisms of action are ineffective efficient in this scenario because the resistance could occur to several groups of herbicides, including never used ones. It is crucial to prioritize rotation and association of herbicides that do not present potential of detoxification by the then known mechanisms. Herbicides that inhibit ALS or ACCase enzymes are more commonly detoxified by plants (Powles and Yu, 2010; Beckie and Tardif, 2012) and, therefore, must be used rationally in the herbicides rotation program. In addition, it will be possible to adopt an alternative management of resistance from the use of synergistic chemicals with herbicides, which are able to inhibit the enzymes responsible for detoxification (Yu and Powles, 2014). The advance in the knowledge of these processes will result on the development of strategy based on the rotation of detoxification mechanisms as a way to prevent evolution of herbicide resistance caused by enhancement degradation.

Final Remarks

Enhanced herbicide degradation is a mechanism of weed herbicide resistance that must be considered an aggravating challenge for weed management and herbicide use. Several species have presented cases of biotypes with multiple resistance caused by degradation enhancement, which reduces the herbicide for weed control.

The knowledge related with the herbicide mechanism of resistance is important for defining adequate prevention and control measures of weed management. The general measures of herbicide resistance prevention could not be adequate for herbicide resistance weeds caused by different mechanisms of resistance, mainly to enhanced degradation. Measures such as application of herbicides in the label rate and in at early stages where the activity of detoxifying enzymes is lower may contribute for the decreasing the evolution of herbicide resistant weeds. Moreover, the use of

synergistic mixtures, including products that inhibit detoxifying enzymes, improves efficiency of herbicides and can be crucial in the prevention of herbicide resistance caused by degradation enhancement. The results of ongoing studies could be used for the developing of more specific measures related to this problem in the near future.

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