Weed resistance to herbicides in Portugal

Resistência Adquirida aos Herbicidas em Portugal

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ABSTRACT

Thirty years ago, the first case of resistance to herbicides was recorded in Portugal: *Alisma plantago-aquatica* resistant to bensulfuron-methyl in rice. Since then, herbicide resistance has been developing in many weed species. Changes in cultural practices, new cropping systems combined with the increased use of herbicides led to the selection of weeds that are resistant to a wide range of herbicides. This problem currently affects our main crops - rice, olive groves, vineyards and orchards (citrus and pomegranate), with one case in maize and another in wheat. There are 16 known weed species (4 dicotyledons and 12 monocotyledons) with resistant populations. Resistance has been confirmed to ALS-inhibiting herbicides (*Alisma plantago aquatica, Cyperus difformis, Echinochloa crus-galli* subsp. *hispidula*, *Echinochloa plyllopogon* and *Papaver rhoeas*), ACCase inhibitors (*E. phyllopogon* and *Leptochloa fusca* subp. *fascicularis*), PSII inhibitors (*Chenopodium album* and *C. difformis*), the herbicide glyphosate, an inhibitor of the EPSPS enzyme (*Conyza bonariensis, C. canadensis, Echinochloa crus-galli, Lolium perenne* and *L. rigidum*) and synthetic auxins (*Papaver rhoeas*). The diversity of physiological and biochemical mechanisms responsible for resistance includes enzyme insensitivity (TSR-Target site resistance), metabolic resistance (NTSR-Non target site resistance) and multiple resistance. This comunication summarises the situation of resistance to herbicides at national level from the perspective of strategies for weed management, taking into account the mechanism of resistance, the availability of alternative herbicides and other cultural practices.

Keywords – TSR, NTSR, site of action, glyphosate, sulfonylureas.

RESUMO

Há cerca de 30 anos foi registado o primeiro caso de resistência adquirida a herbicidas em Portugal: *Alisma plantago-aquatica* resistente ao bensulfurão-metilo na cultura do arroz. Desde então a resistência aos herbicidas tem vindo a desenvolver-se em muitas espécies de infestantes. As alterações nas práticas culturais, nomeadamente novos sistemas culturais, combinados com o aumento da utilização de herbicidas conduziram à selecção de infestantes resistentes a uma vasta gama de herbicidas. Atualmente este problema afecta as nossa principais culturas - arroz, olival, vinha e pomares (citrinos e romãzeiras) havendo a acrescentar um caso na cultura do milho e outro no trigo. Conhecem-se 16 espécies de infestantes (4 dicotiledóneas e 12 monocotiledóneas) com populações resistentes. A resistência foi confirmada a herbicidas inibidores da ALS (*Alisma plantago aquatica*, *Cyperus difformis, Echinochloa crus-galli* subsp. *hispidula, Echinochloa plyllopogon e Papaver rhoeas),* aos inibidores da ACCase, *(E. phyllopogon e Leptochloa fusca* subp. *fascicularis),* aos inibidores do PSII *(Chenopodium album* e *C. difformis),* ao herbicida glifosato, inibidor da enzima EPSPS (*Conyza bonariensis, C. canadensis*, *Echinochloa crus-galli* , *Lolium* perenne e *L. rigidum)* e às auxinas sintéticas *(Papaver rhoeas*). A diversidade de mecanismos fisiológicos e bioquímicos responsáveis pela resistência inclui a insensibilidade da enzima alvo (TSR-*Target site resistance*), a resistência metabólica (NTSR-*Non target site resistance)* e resistência múltipla. Neste artigo apresenta-se de forma resumida a situação da resistência adquirida aos herbicidas a nível nacional numa perspectiva de estratégias adequadas para a gestão das infestantes considerando o mecanismo de resistência, a disponibilidade de herbicidas alternativos e de outras práticas culturais.

Palavras chave – TSR, NTSR, modo de ação dos herbicidas, glifosato, sulfonilureas.

INTRODUCTION

'Weeds, plants that thrive in the face of disturbance, have eluded farmer's attempts at control for over 12,000 years, positioning them as a unique group of extreme stress tolerants. The most successful weeds have a suit of traits that enable them to rapidly adapt to environments typified by stress, growing in hostile conditions or subjeted to massive destruction from agricultural pratices. Throught their ability to persist and adapt weeds illuminate principles of evolution and provide insights into weed management and crop improvement' (Sharma *et al*., 2021). In fact, weed management continues to largely rely on chemical control, creating an unfavourable stress environment for plants but weeds survived and evolve. The evolution of resistance to herbicides is a classic example of weed adaptation (Neve *et al*., 2009). The risk of resistance continues to be high due to the reduction in the diversity of mode of actions and the increase concern on environment and public health concerning herbicides. Sustainable weed management, whether through integrated weed management (IWM) or precision agriculture, does not completely exclude the use of herbicides. That's why knowledge of herbicide properties and resistance mechanisms is a fundamental tool for their effective use, minimising the risk of resistance occurring. Worldwide the number of weed resistance cases continues to increase. To date, a total of 523 cases of resistance to herbicides, from which 272 are dycotiledons species and the remaining 251 monocotyledons (mostly Poaceae). A recent revision of weed resistance in the Iberian Peninsula (Torra *et al*., 2022) indicates a total of eleven cases of herbicide resistance in Portugal and 66 in Spain, involving 33 different weed species – Table 1.

In Portugal, the pesticide resistance situation is most serious for herbicides (ten species, *Alisma plantago aquatica, Echinochloa phyllopogon, E. crus-galli* subsp. *hispidula* and *Cyperus difformis,* in rice; *Chenopodium album* in maize; *Conyza bonariensis; C. canadensis; Lolium rigidum; L. perenne* and *Echinochloa crus-galli* in perennial crops and *Papaver rhoeas* in cereals) followed by insecticides (affecting five species: *Aphis gossipii; Myzus persicae; Leptinotarsa decemlineata; Helicoverpa armigera* and *Cydia pomonella*); and finally fungicides (affecting four species: *Botrytis cynerea/Botryotinia fuckeliana, Plasmopara viticola, Erysiphe necator* and *Venturia inaequalis*) (OEPP, 2023). This paper presents the detailed situation of selected cases of herbicide resistance in Portugal with focus on the mechanisms of resistance including TSR and NTSR and recent cases of multiple resistance.

Table 1 - Herbicide-resistant weeds in Portugal

Year	Species	Mode of action (HRAC code)	Active ingredient	Crop	Region
1995	*Alisma plantago-aquatica*	ALS (2)	bensulfuron-methyl	Rice	Beira Litoral; Ribatejo
2003	*Chenopodium album*	PSII – D1 (5)	atrazina	Maize	Ribatejo
2010	*Conyza bonariensis*	EPSPs (9)	glyphosate	Citrics Olivegrove	Baixo Alentejo
2011	*Conyza canadensis*	EPSPs (9)	glyphosate	Olivegrove	Baixo Alentejo
2013	*Lolium perenne*	EPSPs (9)	glyphosate	Vineyard	Douro
2015	*Echinochloa phyllopogon*	ALS (2)	penoxsulam	Rice	Ribatejo
2015	*Echinochloa crus-galli*	ALS (2)	penoxsulam	Rice	Ribatejo
2018	*Echinochloa crus-galli*	EPSPs (9)	glyphosate	Maize, vineyard, pommegranate	Baixo Alentejo
2019	*Lolium multiflorum*	EPSPs (9)	glyphosate	Vineyard	Douro
2018	*Papaver rhoeas*	ALS (2)	tribenuron-methyl	Wheat	Baixo Alentejo
2020	*Echinochloa phyllopogon*	ACCase (1)	Penoxulam	Rice	Ribatejo
2020	*Echinochloa phyllopogon*	ALS(2)+ACCase (1)	Profoxydim penoxsulam	Rice	Ribatejo
2022	*Papaver rhoeas*	ALS (2)+SAH (4)	tribenuron-methyl + 2,4-D	Wheat	Alentejo
2023	*Cyperus difformis*	ALS(2)+PSII_D1(6)	penoxsulam + bentazone	Rice	Alentejo

CONYZA CANADENSIS (HORSEWEED) RESISTANT TO GLYPHOSATE IN OLIVE GROVES

Conyza bonariensis was first reported to glyphosate in South Portugal in 2010 affecting large areas of intensive olive grove production heavily dependent on herbicides for weed control and in a mixed system of cover cropping in the interrow and chemical control under tree row (Simões *et al.*, 2013). In 2011 resistant populations of *Conyza canadensis* were selected after several years of application of glyphosate from an intensive olive groves also in Alentejo. In order to study the hypothesis of target site resistance related to 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS), shikimate leaf disc assay, sequencing of EPSPS gene and expression levels of the gene encoding this enzyme with real-time PCR were carried out (Domingos *et al*., 2015). After being exposed to glyphosate, one suspected resistant population (B15) showed a lower accumulation of shikimate compared to a reference susceptible population (B). The sequencing of cDNA encoding EPSPS revealed two single nucleotides polymorphisms, but both correspond to silent mutations. The potential mutations, responsible for glyphosate resistance (Pro106Ser/Thr/Ala/Leu; Gly101Ala; Thr102Ile; Pro106Ser /Thr/Gly/ Cys/ Ala/ Ile/ Val/ Met/ Leu, Gly144Asp/Asn e Ala192Thr were not present). The analysis of gene expression also revealed no differences in expression levels of this enzyme, 24-h after glyphosate application. These results suggest that the mechanism of resistance was not target site based because there was no evidence of EPSPS gene mutation or gene over expression in the population studied (Domingos *et al.,* 2015).

ECHINOCHLOA PHYLLOPOGON (LATE WATERGRASS) RESISTANT TO PENOXSULAM IN RICE

Populations of *Echinochloa phyllopogon* (syn. *Echinochloa oryzicola*) resistant to ALS-inhibitor herbicides have been identified in rice fields from three main production regions– north, center and south Portugal. The level of resistance ranges from 10 to 20 however the resistance mechanisms have been not yet fully elucidated. Twenty-one populations were studied under greenhouse and laboratory conditions to confirm whether target site resistance could explain the differences between sensitive and resistant *E. phyllopogon* populations (Mendes *et al*., 2020) In order to confirm this hypothesis, sequencing of acetolactate synthase (ALS-1) gene and the estimation of its number of copies were carried out. After being exposed to penoxsulam, RNA was extracted from R and susceptible populations. The sequencing of cDNA sequence encoding the ALS enzyme revealed that the most frequent point mutations responsible for ALS-inhibitors resistance [Ala122Thr /Pro197Thr/ Ale205Val/ Asp376Glu/ Arg377His/ Trp574Leu/ Ser653Thr/ Gly654Glu] were not present. The estimation of the relative ALS gene copy number, by means of quantitative real time PCR and ΔΔCt value, revealed a two-fold amplification of the ALS-1 gene. The overexpression of the ALS gene contribution as a mechanism of resistance to penoxsulam was not previously mention in the literature (Mendes *et al*., 2020).

PAPAVER RHOEAS (CORNPOPPY) WITH MULTIPLE RESISTANCE TO BOTH ALS-INHIBITOR HERBICIDE (TRIBENURON-METHYL) AND SYNTHETIC AUXIN (2,4-D) IN CEREALS

Multiple resistance mechanisms to ALS inhibitors and auxin mimics in two *Papaver rhoeas* populations from wheat fields from Portugal were. The ALS herbicides suspected was: Bispyribac-sodiuma, Florasulam, Flucarbazone and Imazamox. Dose-response trials, also with malathion (a cytochrome P450 inhibitor), cross-resistance patterns for ALS inhibitors and auxin mimics, alternative herbicides tests, 2,4-D and tribenuron-methyl absorption, translocation and metabolism experiments, together with ALS activity, gene sequencing and enzyme modelling and ligand docking were carried out (Palma-Bautista *et al*., 2020). Results revealed two different resistant profiles: one population (R1) multiple resistant to tribenuron-methyl and 2,4-D, the second (R2) only resistant to 2,4-D. In R1, several target-site mutations in Pro197 and enhanced metabolism (cytochrome P450-mediated) were responsible of tribenuron-methyl resistance. For 2,4-D, reduced transport was observed in both populations, while cytochrome P450-mediated metabolism was also present in R1 population. Moreover, this is the first *P. rhoeas* population with enhanced tribenuron-methyl metabolism. This study reports the first case for *P. rhoeas* of the amino acid substitution Pro197Phe due to a double nucleotide change. This double mutation could cause reduced enzyme sensitivity to most ALS inhibitors according to protein modelling and ligand docking. In addition, this study reports a *P. rhoeas* population resistant to 2,4-D, apparently, with reduced transport as the sole resistance mechanism (Palma-Bautista *et al.,* 2020).

ECHINOCHLOA CRUS-GALLI (BARNYARDGRASS) RESISTANT TO GLYPHOSATE IN A VINEYARD

The levels of resistance to glyphosate of a barnyard grass (*Echinochloa crus-galli*) population collected in a vineyard in Alentejo (South Portugal) was determined in greenhouse and laboratory experiments. Shikimate accumulation fast screening confirmed resistance to glyphosate. Dose-response bioassays highlighted that it was a very resistant (VR) population with a resistance factor (RF=GR_{50} R/GR_{50} S) value of 23. Limited translocation also contributed as a resistant mechanism for this population, where susceptible biotypes showed greater absorption and translocation of ^{14}C-glyphosate compared to the VR population. Enhanced detoxification of glyphosate was confirmed for the first time in *E. crus-galli* where 51% of glyphosate was metabolized to non-toxic products (AMPA and glyoxylate). This VR populations showed 2-fold increase in 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) basal activity and it required 74 times more herbicide to inhibit EPSPS by 50% in relation to the most susceptible population. Additionally, the high glyphosate resistance values at plant (RF, 23) and enzyme level (RF, 74) plus the 2-fold increase in EPSPS basal activity suggests a possible EPSPS overexpression. These results indicated that not only non-target-site resistance (NTSR) mechanisms but also TSR could be implicated in the resistance to glyphosate in *E. crus-galli* (Vázquez-García *et al.,* 2021).

CYPERUS DIFFORMIS (SMALL UMBRELLA SEDGE) WITH MULTIPLE RESISTANCE TO BOTH ALS-INHIBITOR HERBICIDE (PENOXSULAM) AND PSII INHIBITORS (BENTAZONE) IN RICE

A population of *Cyperus difformis* L. (Cyperaceae) from a rice paddy field in Alentejo (South Portugal) was suspected of resistance after several years of application of penoxsulam. Failure of control with bentazone, an alternative mode of action (MoA), raised suspicions of cross-resistance to.to ALS – and PSII-inhibiting herbicides. In order to detect herbicide resistance to both active substances, a qualitative seedling-based quick-test was carried out using seeds of this population collected in 2020, and three susceptible populations. After seed germination, seedlings at the 1st leaf stage were transplanted to ELISA plates, 10 per well, in 5 mL of agar medium (1 %) under conditions of alternation of temperature and light in a growth chamber. A range of five concentrations was used for resistance screening (0; 0.1; 0.5; 1;5;10 mg L^{-1}). When 5 mg L^{-1} penoxsulam or bentazone were added, susceptible plant growth stopped, and the 1st leaf turned chlorotic or necrotic while the resistant populations remained green and continued developing new leaves. The suspected population was compared with the susceptible populations. Plants of the former developed rapidly, with only about 14 days needed to finish the test. A non-parametric test allowed to significantly separate resistant from susceptible populations at a discriminant concentration of 5 mg L^{-1} for both herbicides. Further validation with pot experiments is ongoing. There is strong evidence that the studied population of *C. difformis* is resistant both to penoxsulam and bentazone. There are few cases of resistance to bentazone worldwide, therefore this would encompass a unique case of cross resistance to both MoA in *Sagittaria montevidensis* from Brasil. This is the first report of bentazone resistance in Europe (Santos *et al.,* 2022).

CONCLUSION

Metabolic resistance was first reported in Portugal recently in three different weed species and another one with multiple resistance to two different mode of action. These mechanisms of resistance limit the use of alternative chemicals for weed control. In the near future, weed management will have to be framed by a better conception of cropping systems (agroecology) and no longer be considered a problem that can only be solved with the application of herbicides (Mortensen *et al.*, 2000).

REFERENCES

Domingos, D.; Rodrigues, M.; Andrade, E.; Monteiro, A. & Calha, I.M. (2015) - Genetic characterization of EPSPS from *Conyza canadensis* resistant to glyphosate. *In: Proceedings of 17th European Weed Research Society Symposium* "Weed management in changing environments", Montpellier, France, p.106.

Mendes, A.; Dias, M.; Monteiro, A.; Andrade, E. & Calha, I.M. (2020) - Weed resistance status in *Echinochloa phylopogon* from portuguese rice fields. *In: Proceedings of 8th International Weed Science Congress "Weed Science in a Climate of Change"*, Bangkok, Thailand.

Mortensen, D.A.; Bastians, L. & Sattin, M. (2000) - The role of ecology in the development of weed management systems: an Outlook. *Weed Research*, vol. 40, n. 1, p. 49-62. https://doi.org/10.1046/j.1365-3180.2000.00174.x

Neve, P.; Vila-Aiub, M. & Roux, F. (2009) - Evolutionary-thinking in agricultural weed management. *New Phytologist*, vol.184, p. 783–793. https://doi.org/10.1111/j.1469-8137.2009.03034.x

OEPP (2023) - *EPPO Database on Resistance Cases*. [cir. 2024.01.05] https://resistance.eppo.int/

Palma-Bautista, C.A.; Portugal, J.; Vazquez-García, J.G.A.; Osuna, M.D.; Torra, J.; Lozano-Juste, J.; Gherekhloo, J. & De Prado, R (2020) - Tribenuron-methyl metabolism and the rare Pro_{197}Phe double mutation together with 2,4-D metabolism and reduced absorption can evolve in *Papaver rhoeas* with multiple and cross herbicide resistance to ALS inhibitors and auxin mimics. *Pesticide Biochemistry and Physiology*, vol. 188, art. 105226. https://doi.org/10.1016/j.pestbp.2022.105226

Santos, J.; Aguiar, F.C. & Calha, I.M. (2022) - *Cyperus difformis* cross-resistance among rice herbicides in South Portugal. *In: Abstracts 19th EWRS Symposium*, Athens, Greece, p.102.

Sharma, G.; Barney, J.N.; Westwood, J.H. & Haak, D.C. (2021) - Into the weeds: new insights in plant stress. *Trends in Plant Science*, vol. 26, n.10, p. 1050–1060. https://doi.org/10.1016/j.tplants.2021.06.003

Simões, M.; Portugal, J.; Monteiro, A. & Calha, I.M. (2013) - Citrus orchard with *Conyza bonariensis* resistant to glyphosate: 211–216. *In: Libro de Actas del XIV Congreso SEMh, Sociedad Española de Malherbología,* Valencia, Spain, p. 211-216.

Torra, J.; Montull, J.M.; Calha, I.M.; Osuna, M.D.; Portugal, J. & de Prado, R. (2022) - Current Status of Herbicide Resistance in the Iberian Peninsula: Future Trends and Challenges. *Agronomy*, vol. 12, n. 4, art. 929. https://doi.org/10.3390/agronomy12040929

Vázquez-García, J.G.; Rojano-Delgado, A.M.; Alcántara-de la Cruz, R.; Torra, J.; Dellaferrera, I.; Portugal, J. & De Prado, R. (2021) - Distribution of glyphosate-resistance in *Echinochloa crus-galli* across agriculture areas in the Iberian Peninsula. *Frontiers in Plant Science*, vol. 12, art. 617040. https://doi.org/10.3389/fpls.2021.617040