Metabolic resistance to pre-emergence herbicides in grasses

Metabolische Resistenz gegen Vorauflaufherbizide bei Gräsern

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DOI 10.5073/jka.2020.464.076

Abstract

Black-grass (*Alopecurus myosuroides* Huds.) and rye-grass (*Lolium* spp) are very competitive grass weeds. During the last decades, they have evolved resistance to post-emergence herbicides, in particular ACCase- and ALS-inhibitors. This requires more complex weed management strategies. To ensure good control of both weeds, pre-emergence treatments become increasingly important. Particularly flufenacet has become a key herbicide for the control of multiple-resistant black-grass and rye-grass. Yet, in some of those populations, reduced flufenacet efficacy start to be observed. In a screening with black-grass and rye-grass populations of worldwide origins, most populations could be controlled with the registered field rate of flufenacet, however differences in the level of flufenacet efficacy were observed and were in correlation with enhanced flufenacet metabolism. This was particularly the case for rye-grass populations. The use of flufenacet in mixtures with diflufenican, particularly in combination with flurtamone or metribuzin or aclonifen, improved the efficacy significantly. In decreased flufenacet sensitive populations, the efficacy of other pre-emergence herbicides like pendimethalin, prosulfocarb, S-metolachlor, dimethenamid-P and pethoxamid, was also significantly decreased whereas other herbicides like pyroxasulfone or diflufenican remained highly active. This decreased efficacy of flufenacet as well as S-metolachlor was associated with higher metabolism involving glutathione-s-transferases. Although differences between populations were sometimes relatively small, best weed management practices (e.g. application of full dose rates and mixtures) should be applied to reduce selection pressure and prevent the development of resistance. This is particularly important as flufenacet is one of the few still active herbicides suitable for the control of multiple-resistant grass weeds. Use of mixtures associated with agronomic solutions in an Integrated Weed Management approach has to be the main approach.

Keywords: Weed resistance, herbicide, enhanced metabolism, flufenacet, black-grass, rye-grass, glutathione transferases

Zusammenfassung

Acker-Fuchsschwanz (*Alopecurus myosuroides* Huds.) und Weidelgras (*Lolium* spp) sind sehr konkurrenzstarke Ungräser. In den letzten Jahrzehnten haben diese Arten Resistenzen gegen im Nachauflaufeingesetzte Herbizide, insbesondere ACCase- und ALS-Hemmer, ausgebildet. Daher sind für die Bekämpfung dieser Arten komplexe Management-Strategien nötig. Vor diesem Hintergrund hat der Einsatz von Vorauflauf-Herbiziden deutlich an Bedeutung gewonnen. Insbesondere der Einsatz von Flufenacet hat sich zu einem wichtigen Teil der Bekämpfungsstrategie von Acker-Fuchsschwanz und Weidelgras mit multiplen Resistenzen entwickelt. Jedoch wurde in einzelnen dieser multiple-resistenten Populationen eine reduzierte Wirkung von Flufenacet festgestellt. In einem Screening-Versuche mit verschiedenen Populationen von Acker-Fuchsschwanz und Weidelgras aus der ganzen Welt konnte die Mehrzahl der Populationen mit der zugelassenen Aufwandmenge von Flufenacet ausreichend bekämpft werden. Es wurden aber Unterschiede in der Wirksamkeit von Flufenacet festgestellt, die mit einer erhöhten Metabolisierung von Flufenacet zusammenhingen. Diese Korrelation wurden insbesondere bei Weidelgräsern beobachtet. Der Einsatz von Flufenacet in Mischungen mit Diflufenican, insbesondere in Kombination mit Flurtamone oder Metribuzin oder Aclonifen, erhöhte die Wirksamkeit signifikant. Bei Populationen mit einer verminderten Flufenacet-Sensitivität zeigten auch andere Vorauflauf-Herbizide wie Pendimethalin, Prosulfocarb, S-Metolachlor, Dimethenamid-P und Pethoxamid eine verminderte Wirksamkeit während andere Herbizide wie Pyroxasulfone oder Diflufenican weiterhin eine hohe Wirksamkeit zeigten. Die verminderte Wirkung von Flufenacet und S-Metolachlor war mit einer erhöhten Metabolisierung durch Glutathione-S-Transferasen assoziiert. Auch wenn die Unterschiede zwischen den Populationen in einigen Fällen gering waren, sollten effektive Management-Strategien wie die Applikation der vollen Aufwandmenge und von Mischungen angewendet werden um den Selektionsdruck zu vermindern und die Entwicklung von Resistenzen zu verhindern. Dies ist vor allem deswegen so entscheidend, weil der Wirkstoff

Flufenacet einer der wenigen Wirkstoffe ist, der zur Bekämpfung von Ungras-Populationen mit multipler Resistenz eingesetzt werden kann. Die Anwendung von Mischungen in Kombination mit ackerbaulichem Maßnahmen sollte im Rahmen eines integrierten Konzeptes zur Ungrasbekämpfung eingesetzt werden.

Stichwörter: Acker-Fuchsschwanz, erhöhter Metabolismus, Flufenacet, Glutathion-Transferasen, Herbizidresistenz, Herbizide, Weidelgras

Introduction

In the last decades, herbicide resistance has become a major issue for many weeds (Powles and Yu 2010, Gressel 2009; Peterson et al., 2018), in particular grass weeds like black-grass and rye-grass. The use of pre-emergence herbicides has gained more and more importance due to the evolution of resistance in these grass weeds to herbicides such as inhibitors of ACCase (HRAC group A) and ALS (HRAC group B) applied post-emergence in late winter or early spring. The registration evolution with a decrease in approved active ingredients, or lower registered dose rates, as well as the lack of innovation of chemistries with novel modes of action, have impacted on the weed control strategies with increased use of autumn pre-emergence herbicides (HRAC K3, F1, N, …) and therefore elevated the selection pressure on herbicides resistance. In that context, it is important to follow carefully the resistance evolution to these herbicides in order to continue to bring farmers efficient solutions to keep the weed pressure low and maintain reasonable crop yields.

We report the evolution of resistance against autumn-applied herbicides (mainly applied pre-emergence) involved in the control of grasses, in particular black-grass and rye-grass in cereal-based cropping systems. The implication of glutathione-s-transferase in the detoxification of flufenacet and S-metolachlor will be described. This knowledge allows to set new solutions for autumn grass weed control, based on mixtures of flufenacet, diflufenican and a third partner, either metribuzin or aclonifen (MATENO®). Aclonifen, with a new mode of action used in this weed control segment, is a new tool in terms of resistance management. Combined with agronomic solutions, it increases the toolbox of Integrated Weed Management solutions.

Material and Methods

Plant culture and herbicide assays (dose response analyses)

Several tens of rye-grass and black-grass populations collected worldwide were treated with different pre-emergence herbicides containing in particular flufenacet (CADOU (FFA solo)) and flufencet and diflufenican in combination (FOSBURI) as described previously (Duecker et al. 2019 a; Duecker et al. 2019 b). The effective dose to reduce the plant growth by 50% and 90% have been determined and compared to the recommended field dose. Representative data are presented in Figures 1 and 2.

Detoxification of flufenacet *in planta*

Five days plantlets were treated with flufenacet labelled with ^{14}C. After extraction, both the parent compound and its metabolites were analyzed by HPLC and identified by LC-MS/MS as described by (Duecker et al. 2019 a; Duecker et al. 2019 b). The flufenacet degradation rate was reported for different populations as well as the detoxification pathway (Figures 3 and 4).

Results and discussion

Dose response of several pre-emergence herbicides on rye-grass populations

Three rye-grass populations sensitive to flufenacet and three resistant populations were selected to study their sensitivity to several pre-emergence herbicides as reported in Figure 1. These data showed that other K3 inhibitors, chemically different, like pyroxasulfone, can still control all populations efficiently. This suggests that the mechanism of resistance can be non-target site related. In addition, an herbicide with another mode of action, like diflufenican (DFF), often mixed with flufenacet, stayed fully active on all populations tested.

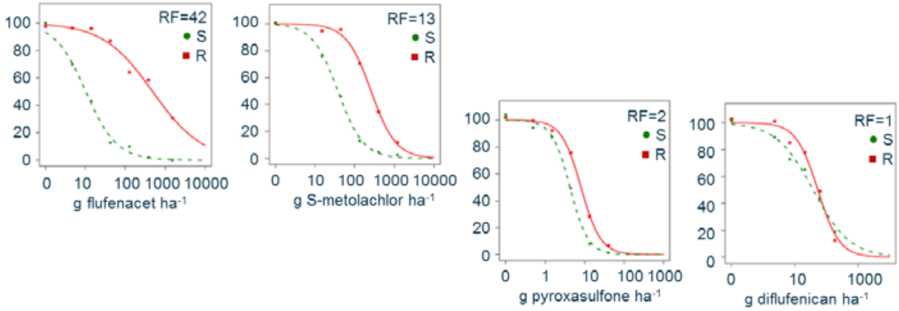

Fig. 1 Example of dose response of different active ingredients applied in pre-emergence on rye-grass. The data represent the average of three resistant populations (red, solid line) and three sensitive populations (green, dotted line). RF: resistance factor.

Abb. 1 *Beispiel von Dosis-Wirkungs-Kurven verschiedener Vorauflaufherbizide zur Bekämpfung von Weidelgras. Die Daten stellen die Mittelwerte von drei resistenten (rote, durchgezogene Linie) und drei sensitiven (grüne, gestrichelte Linie) Populationen dar. RF: Resistenzfaktor.*

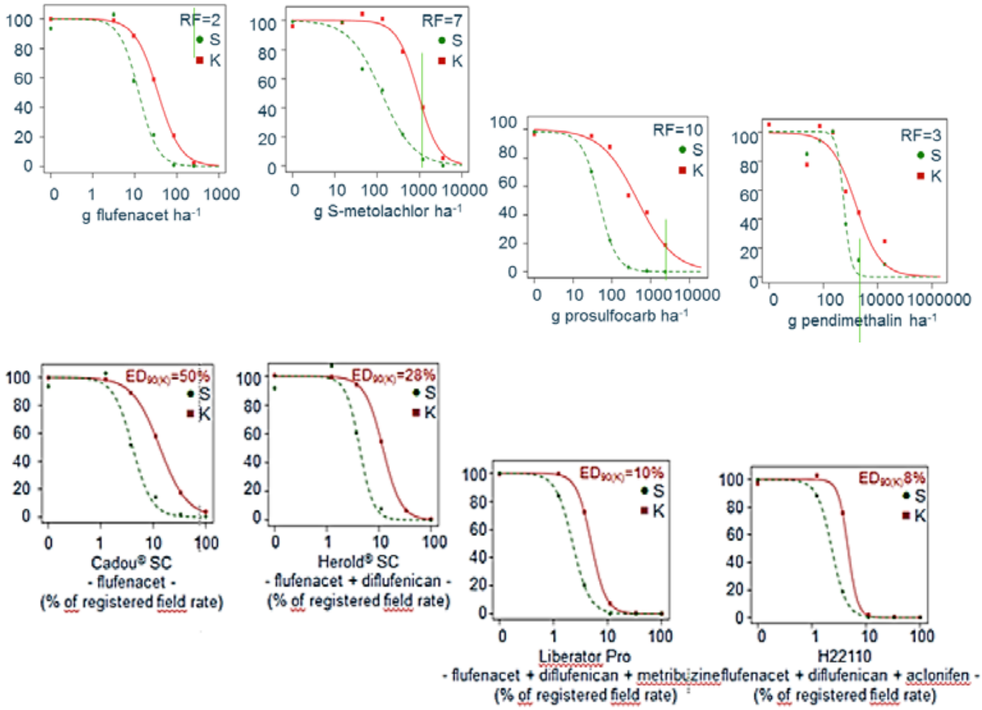

Fig. 2 Example of dose response of different active ingredients applied in pre-emergence on black-grass. The data represent the average of three resistant populations (red, solid line) and three sensitive populations (green, dotted line). RF: resistance factor.

Abb. 2 *Beispiel von Dosis-Wirkungs-Kurven verschiedener Vorauflaufherbizide zur Bekämpfung von Acker-Fuchsschwanz. Die Daten stellen die Mittelwerte von drei resistenten (rote, durchgezogene Linie) und drei sensitiven (grüne, gestrichelte Linie) Populationen dar. RF: Resistenzfaktor.*

The same approach was followed using black-grass with the difference that fewer populations showed a decreased sensitivity to flufenacet with a resistance factor of only 2 compared to 42 for rye-grass (Figs. 1 and 2). The data obtained showed that in comparison to S-metolachlor, prosulfocarb and pendimethalin, flufenacet remains the most active herbicide on black-grass (Fig. 2, upper data set). To compare the efficacy of mixtures (Figure 2, bottom data set), the recommended field dose is represented by 100 (100%) on the x-axis. A dose of 90% of flufenacet controlled only 50% (ED90(K) = 50%) of less sensitive black-grass populations (red, solid line). A mixture of flufenacet and DFF already allowed controlling 28% of these populations with a dose of 90% (ED90). Finally, three-way mixtures, either with metribuzine or aclonifen restored the control of all populations tested. This showed that to mitigate the evolution of resistance as much as possible, flufenacet has to be mixed with DFF and a third partner, either metribuzin or aclonifen. These mixtures contain active ingredients representing three modes of action.

Detoxification of flufenacet *in planta*

A time course of flufenacet content in three sensitive and three resistant rye-grass populations was performed. Rye-grass populations resistant to flufenacet (red lines, Fig. 3) showed a much faster detoxification of flufenacet than the sensitive populations (green lines, Fig.3). In addition, a good correlation was found between the rate of flufenacet degradation and its biological efficacy found in the greenhouse (Fig. 3). This suggests that flufenacet metabolism is the main mechanism causing flufenacet resistance. Similar results (not shown) were found in black-grass.

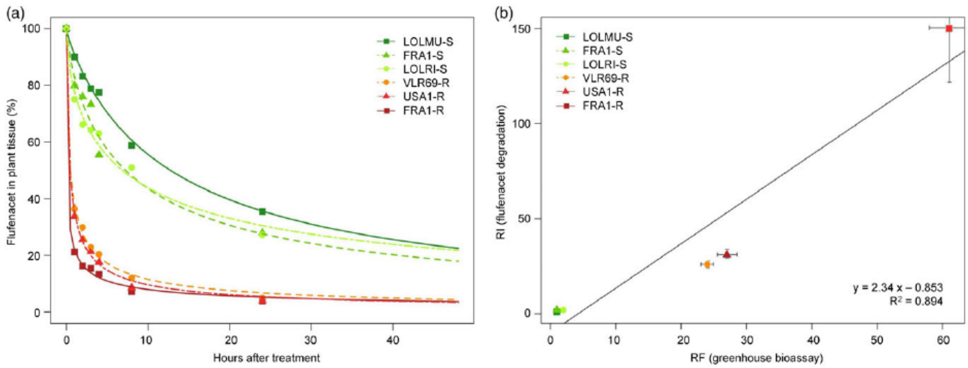

Fig. 3 Detoxification rate of flufenacet (a) in three resistant (red) and susceptible (green) populations of rye-grass. Comparison of the detoxification rate with the resistance factor (RF) determined in greenhouse bioassays (b).

Abb. 3 *Detoxifikationsrate von Flufenacet (a) in drei resistenten (rot) und sensitiven (grün) Weidelgras-Populationen. Vergleich der Detoxifikationsrate mit den im Gewächshaus-Biotest ermittelten Resistenzfaktoren (RF) (b).*

Using HPLC and LC-MS/MS, the different flufenacet metabolites were characterized. It was found that the first metabolite formed was a conjugate between glutathione and flufenacet suggesting a strong involvement of glutathione-s-transferases. Then, different secondary metabolites were found as summarized in Figure 4.

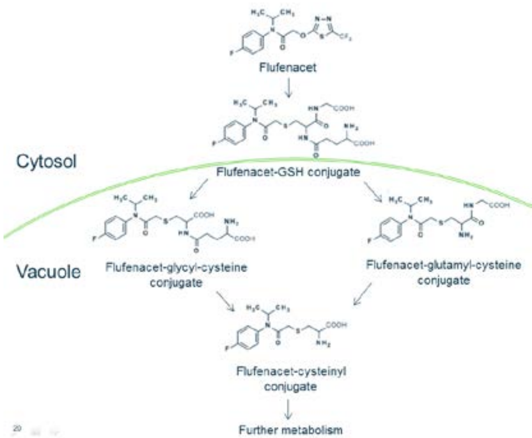

Fig. 4 Summary of flufenacet degradation pathways evidenced by HPLC and LC-MS / MS analyses in rye-grass and black-grass.

Abb. 4 *Zusammenfassung des Degradationspfades von Flufenacet in Weidelgras und Acker-Fuchsschwanz (analysiert mit Hilfe von HPLC und LC-MS / M).*

Discussion and Conclusion

In a few populations of rye-grass and black-grass, a decreased sensitivity to flufenacet was observed with a higher frequency in rye-grass than in black-grass. Because flufenacet resistance was so far observed only in rare cases in agronomic situations, it is important to decrease the selection pressure as soon as possible. In addition to combinations with agronomic solution (crop rotation, proper soil management, delayed sowing date, false seed bed, etc.) one of the best approaches is to combine flufenacet with other pre-emergence herbicides with other modes of action like DFF and metribuzine or aclonifen. These solutions, as for example, Mateno®, have proven an optimal efficacy on all tested populations so far. Pre-emergence herbicides are essential tools to control grass weeds and have to be used carefully to mitigate resistance evolution. Flufenacet was found to be faster detoxified in less sensitive population, involving glutathione-s-transferases (GSTs). Genomic approaches will be useful to characterize what gene(s) encoding GSTs will be involved (Ravet et al., 2018). This finding allows setting biochemical and molecular tools to perform diagnostics of the evolution of resistance as early as possible and thus define the best active ingredient combinations to obtain and maintain a high efficacy as long as possible. Combined with Integrated Weed Management, there are opportunities to maintain efficient weed control and insure reasonable crop yield production.

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